Inflammation in Schizophrenia: The Contribution of Inflammatory Markers to the Emergence of Negative Symptoms, A Systematic Review and Meta-Analysis

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Abstract

Schizophrenia is a highly debilitating disorder that affects up to 20 million individuals worldwide, characterised by severe positive (hallucinations, delusions), negative (anhedonia, avolition), and cognitive (impairment) symptoms. Negative symptomology is broadly defined as a 'loss of function', including deficits in affective responses that are notoriously long-lasting, difficult to treat, and underpin concerning reductions in quality of life. The mechanisms underlying schizophrenia development remain poorly understood, however contemporary research suggests inflammatory abnormalities and alterations in immune functioning are crucial in both the development of disease, and emergence of negative symptomology in particular. Indeed, perturbations in peripheral inflammatory cytokine concentration have been reported across the whole schizophrenia spectrum, alongside elevated markers of central glial activation which is indicative of neuroinflammation in schizophrenia. This is proposed to cause excessive generation of neuroactive metabolites in the brain that may facilitate dysregulation of dopaminergic, glutamatergic, and serotonergic neurotransmission within the central nervous system. Further, chronic low-grade inflammation may impair sufficient activation and connectivity of brain areas associated with affective functioning, and contribute to pathogenic processes that foster the development of negative symptoms in schizophrenia. However, to our knowledge the prospective relationship between cytokine perturbations and severity of negative symptoms has never been assessed via systematic review and meta-analysis.

The aim of this study was to provide an up-to-date overview of cytokine perturbations, and the prospective relationship between inflammatory cytokine concentration and negative symptom severity a in first episode, neuroleptic naïve schizophrenia population. Following PRISMA guidelines, a systematic literature search was conducted using keywords which were adjusted to subject headings appropriately across EMBASE, MEDLINE, and PsychInfo

databases. This primary search identified 1,360 records, which were screened for eligibility against prespecified inclusion criteria (published between 1982 – 2020, first episode, drug naïve psychosis patients, within first five years of illness, assessing circulating cytokines and negative symptoms), whereby 10 studies were included in the final systematic review and random-effects, pooled-effect meta-analysis. A narrative review was conducted to assess the relationship between cytokine concentration and severity of negative symptoms, due to lack of sufficient supplementary data supplied by corresponding authors and absence of correspondence upon request, which obstructed any larger scale analyses taking place.

These studies reported data on 651 first-episode psychosis patients, 521 healthy control subjects, 10 inflammatory cytokines, and negative symptoms as assessed by the PANSS or SANS scale. A significant effect size was reported for IFN-γ, IL-6, IL-12, and IL-17 ($p = <0.05$), suggesting widespread perturbation of multiple cytokines in early schizophrenia pathology. In terms of cytokine relationship with negative symptom severity, significant positive relationships were described for the PANSS negative subscale and IL-1β, IL-2, IL-4, IL-6, and TNF-α, across a total of five different studies. Furthermore, significant negative relationships were described for the PANSS negative subscale and IL-10 in two studies. These results display clear evidence of perturbed immune functioning in early psychosis on a large scale, alongside promising findings from individual cohort data that suggest an interaction of perturbed cytokines with subsets of negative symptomology specifically. Importantly, the current systematic review utilised multiple aspects of the varying definitions of schizophrenia in order to synthesise data on a group devoid of apparent comorbidities, consequences of ageing and disease progression, and antipsychotic medication that may obscure findings with regards to cytokine concentration and symptomology. Future research should seek to come to an agreement about which inflammatory markers may contribute most to negative symptom emergence, acknowledge a common method for cytokine quantification,

and provide extensive, longitudinal data on negative symptom severity to allow for more effective analysis of causality in schizophrenia pathology.

Contents

1.0 Introduction

1.1 Characteristics of Schizophrenia

Schizophrenia (SCZ) is a highly debilitating neurodevelopmental disorder that affects up to 20 million individuals globally. SCZ is associated with a reduced quality of life, severe cognitive dysfunction, and increased mortality (Weinberger, 1987, James et al., 2018). Symptoms of SCZ typically present during the adolescent period, and manifest as positive symptoms (hallucinations, delusions), cognitive symptoms (memory impairment, disorganised thought), and negative symptoms (anhedonia, anergia, apathy). Due to the aetiological heterogeneity that is present in SCZ, the disorder remains relatively poorly understood, and effective therapeutic treatment for the alleviation of SCZ symptoms is currently lacking. Concerningly, SCZ contributes to an enormous economic burden, summating to £2.2 billion in England in 2007, and is estimated to cost up to £3.7 billion by 2026 (NICE, 2014). Furthermore, SCZ ranked as the 12th most debilitating disorder worldwide in a review of the 'Global Burden of Disease' conducted in 2016 (Vos et al., 2017).

Typically early in SCZ progression, a 'prodromal' period occurs in which an individual is considered 'at risk' of developing psychosis. Prodrome may consist of nonspecific changes in behaviour, detachment, difficulty with communication and attention, and general impairments in personal and social functioning. This often occurs simultaneously with forms of delusional thought processes, and these symptoms may affect an individuals' capability to maintain occupational and educational roles, as well as interpersonal relationships (Larson et al., 2010, Health, 2014). Subsequent to the prodromal period, an individual may experience a first episode of psychosis (FEP), which is comprised of severe positive, negative, and

cognitive symptoms that can persist indefinitely (Yung et al., 2004, Health, 2014). The transition to FEP and SCZ signifies increased intensity of psychotic symptoms that require medical attention (Larson et al., 2010). This is defined most recently by the 'Diagnostic and Statistical Manual of Mental Disorders' (DSM) as the emergence multiple positive, negative, or cognitive symptoms which occur for a substantial period of time (over 1 month). This definition also states that speech abnormalities, hallucinations, or delusions must be present as part of the symptomology for the diagnosis of FEP (Diagnostic, 2013). It is estimated that over one third of individuals classified as 'clinical high-risk of psychosis' experiencing prodromal symptoms, eventually transition to the diagnosis of psychosis across a period of 3 years (Fusar-Poli et al., 2012). This diagnosis usually represents a degree of psychotic symptoms that may affect an individual across their entire lifespan, and an alarming reduction in life expectancy of up to 20 years (Laursen et al., 2014). High mortality rates that are evident in SCZ are suggested to be a result of multiple factors including poor lifestyle choices, poor dietary habits, high prevalence of tobacco and cannabis smoking, which may also contribute to associated comorbidities in SCZ (De Leon and Diaz, 2005, Green et al., 2005, Laursen et al., 2012). Furthermore, within the SCZ population there is a substantially increased risk of suicide (Saha et al., 2007).

1.2 Nature vs Nurture

The mechanisms underpinning the pathophysiology of SCZ are largely unknown, with various factors thought to contribute to the complexity of the disorder. However, it is relatively well established that genetic alterations and environmental stressors are salient in the development of FEP and eventual SCZ. The neurodevelopmental hypothesis of SCZ suggests that alterations in genetic coding during development may predispose individuals to

the later manifestation of disease, through maldevelopment of specific signalling pathways that increase vulnerability to stress. Added to this, environmental stressors that can occur prenatally (infection, birth complications, etc.) and perinatally (trauma, neglect, child abuse, etc.) can cause the sensitisation and priming of immune cells, which may further increase vulnerability to stress during adolescence. Consequently, stressors that occur later in life can trigger defective immune-inflammatory responses that underpin the progression of SCZ pathology (Fatemi and Folsom, 2009, Karl and Arnold, 2014, Müller, 2018). For example, there is evidence to suggest that *in utero* infection (influenza, maternal infection, respiratory tract infections) combined with the apparent genetic alterations cause an increased risk of later SCZ development in offspring (Bassett et al., 2010, Khandaker et al., 2013, Watkins and Andrews, 2016). Contemporary clinical research proposes a 'two-hit' hypothesis in which these environmental 'hits' and genetic alterations exhibited prenatally and perinatally, combine with later life environmental 'hits' that culminate in disease presentation, discussed later (Gogtay et al., 2011, Müller, 2018, Moslem et al., 2019). Large bodies of evidence surrounding SCZ progression suggest that excessive stress during key developmental periods are important for SCZ manifestation, and that these stressors may interact with other factors to cause the development of pathogenic illness (Weinberger, 1987, Gogtay et al., 2011). This appears to be rooted partly in defective immune responses, in which the SCZ population display hyperactivation of innate immune cells that cause chronic upregulation of proinflammatory cytokines. This can elicit tissue damage and lead to persistent oxidative stress (Bitanihirwe and Woo, 2011, Müller, 2018).

Multiple meta-analyses have identified increased markers of oxidative stress (Bitanihirwe and Woo, 2011, Pedrini et al., 2012), as well as chronic neuroinflammation and cytokine action in SCZ (Miller et al., 2011, Goldsmith et al., 2016). Consequently, defective inflammation can elicit even further oxidative stress, tissue damage, and neurodegeneration that may contribute

to SCZ pathology through the impairment of different signalling cascades, which may lead to presentation of negative symptoms in particular (Bitanihirwe and Woo, 2011, Müller et al., 2015). However, the potential relationship between inflammation and negative symptoms has been largely neglected until recent years. It is important to note that SCZ is an enormously heterogeneous disease, and changes that occur may differ on a patient-to-patient basis dependent on duration of untreated psychosis, severity of environmental stressors that are experienced, genetic factors, comorbid drug use, and medication status, all of which can influence the nature and extent of negative symptoms that are present (Health, 2014, Correll and Schooler, 2020).

1.3 Negative Symptoms in FEP & SCZ

The negative symptoms in SCZ have been classically described as a 'loss of function' involving the absence of drive, interest, and deficit in affective responses (Andreasen, 1982), and can be categorized in order to identify specific symptomatology. Negative symptoms can emerge at any time throughout the duration of disease, and have been reported to be experienced by over 90% of patients upon their first presentation to a clinical setting (an der Heiden et al., 2016). These can be classified as primary negative symptoms which are intrinsic to SCZ pathology, or secondary symptoms which may present as a side effect of other primary symptoms. For example, the emergence of secondary negative symptoms as a consequence other psychotic symptomology, comorbidities, or medication status (Kirschner et al., 2017). Specifically, these symptoms are grouped by the DSM into two separate subdomains, the first concerning 'diminished expression'. This subdomain refers to phenomena of blunted affect (difficulty in articulation of emotion) and alogia (poverty of speech) which present overall as deficits in expression and loss of emotional range. The

second subdomain is concerned with ‘avolition-amotivation’ symptoms that may present as asociality (avoidance of social interaction) and motivational anhedonia (lack of goal directed behaviour) (Association, 2013). Specifically, the latter subdomain has been suggested as a significant negative symptom construct that is present as early as the prodromal period, and may underpin the reduction in functioning that is exhibited as a consequence (Foussias and Remington, 2010).

Negative symptomology is notoriously longer lasting than other psychotic symptoms, and is associated with a significantly reduced quality of life and social functioning (Eack and Newhill, 2007, Robertson et al., 2014). These symptoms are frequently described as the first type of SCZ symptoms that are experienced, and are associated with the transition from ‘high risk’, to psychosis and SCZ (Correll and Schooler, 2020). SCZ Patients that exhibit long and enduring negative symptoms are often classified as exhibiting ‘deficit syndrome’ SCZ, which was most recently reported by meta-analysis as present in approximately 33% individuals diagnosed with SCZ (López-Díaz et al., 2018). Concerningly, a separate meta-analysis conducted by Fusar-Poli (2015) suggested that existing treatments for SCZ symptomology displayed minimal efficacy as a means of improving negative symptoms that are experienced. This suggests a caveat in the overall effectiveness of current treatment, whereby even after therapeutic medication, unrelenting negative symptoms may still exert devastating effects with regards to mood and quality of life. Chronically, this may manifest as a lack of motivated behaviour, reward processing impairments, and emergence of anhedonic functioning that may underpin these reductions in quality of life (Correll and Schooler, 2020).

The pathological mechanisms of action that underpin negative symptoms in SCZ may have the potential to trigger comorbidities of major depression through shared biological pathways and symptom presentation, making accurate isolation and assessment of negative symptoms more difficult to achieve (Goldsmith and Rapaport, 2020). Comorbid depression can further

exacerbate the severity of ill-health, contribute to more severe reductions in quality of life, and has been suggested as present in approximately 40% of individuals with SCZ across the lifespan (Huppert et al., 2001, Conley et al., 2007). As illustrated in Figure 1, individuals diagnosed with comorbid depression in SCZ are reported to exhibit lower satisfaction with life, reduction in mood, anhedonia, and suicidal thought processes, highlighting the similarities that depression may have with negative symptoms (Upthegrove et al., 2017). Furthermore, a recent meta-analysis conducted by Krynicki et al. (2018), also suggested that alongside potential overlapping biological pathways, individuals with depression display overlapping symptomatology with individuals exhibiting severe negative symptoms, through expression of anhedonia, anergia, amotivation, asociality, and avolition. This review suggested that these symptoms may present as features of both depressive and negative symptomology, highlighting the potential of symptoms to overlap, and therefore making negative symptoms increasingly difficult to identify. Other review articles have suggested that positive symptom exacerbation during a psychotic episode, as well as side-effects of therapeutic treatment can conceal the presentation of negative symptoms, adding to the complexity of disentangling and defining primary negative symptoms (Möller, 2007).

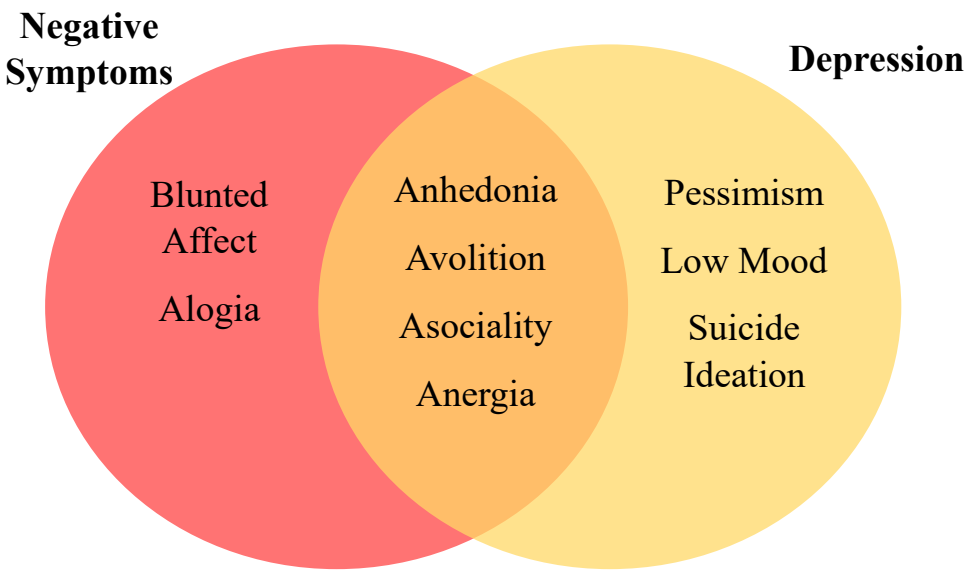


Figure 1. A Venn Diagram displaying symptomology classified as a 'negative symptom' or a 'depressive' symptom. The middle section represents symptoms that may span both negative and depressive behaviour.

Therefore, highly sensitive symptom severity scales are used in practice in order to provide an objective representation of the positive, negative and cognitive symptoms experienced. The main clinical tool used to assess these symptoms in SCZ research is the 'Positive and Negative Symptom Scale' or 'PANSS Questionnaire'. This questionnaire consists of 30 items in order to classify a patient in terms of their symptomology, and is split into subsets of positive, negative, and cognitive scales (Kay et al., 1987). Alternatively, the 'Scale for Assessment of Negative Symptoms' or 'SANS' can be used in order to identify and isolate negative symptoms of SCZ, as this scale exclusively measures subscales of affective flattening, alogia, avolition-amotivation, anhedonia, and asociality (Andreasen, 1982). Finally, given that multiple symptoms span both negative and depressive domains, the 'Calgary Depression Scale for Schizophrenia' has since been implemented in order to sensitively categorise depressive symptoms more specifically and separately to negative symptomatology (Addington et al., 1996). For example, anhedonia has now been further separated into 'consummatory' anhedonia (lack of pleasure in current activity) which applies more to depressive domains, whereas 'motivational' anhedonia (lack of anticipation of pleasure in future events) relates more closely to negative symptomology in SCZ (Krynicki et

al., 2018). These scales allow for classification of individuals based on their symptomology in order to determine suitable therapeutic treatment required. However, it must be acknowledged that the accurate classification of patient symptoms is largely reliant on the capability of the individual conducting the clinical assessment, as different symptoms may overlap to promote a crippling disease state. Furthermore, the use of various symptom scales alongside potential differences in interpretations of behaviour by clinicians create increasingly 'blurred lines' for the genuine definition and classification of negative symptoms (Kumari et al., 2017).

It is increasingly important to recognise that negative symptoms are a distinct category based on behavioural alterations in patients, and represent a measurable aspect of disease that is a product of specific biochemical/physiological changes that are occurring in the brain and central nervous system (CNS) to cause the emergence of negative symptoms in particular. Therefore, the measurement of these symptoms separately via specific rating questionnaires in order to determine symptomology severity, accentuates that the negative aspects are an important and distinct subcategory that are essential in any diagnosis of disease (Blanchard et al., 2020). This may be difficult due to the obscure presentation of negative symptoms and the nature in which they are assessed. For instance, alogia is broadly defined as 'poverty' or 'absence' of speech and elaboration. Therefore, misidentification of the extent and severity of negative symptoms may occur, as lack of engagement means the clinician must rely largely on behavioural tendencies and brief responses to questions during assessment (Marder and Galderisi, 2017).

Often, clinicians prioritise the treatment of psychotic symptoms as these are reported to result more often in hospital admission, through acute presentation of symptoms that are more severe, disturbing, and afflicting than negative symptoms (Hanson et al., 2010). This is because acute psychotic episodes are characterised by manic positive symptoms associated

with huge increases in risk of harm to the self and others (Haddock et al., 2013). Furthermore, clinicians have been reported to focus on 'textbook' outcomes of a reduction in pathology via symptomology rating scale reductions, and improvements in cognition, whereas patients may place more emphasis on 'functional' outcomes of improvements in satisfaction, productivity, and regulation of emotions (Lloyd et al., 2017). As a result, this places a larger burden on patients who battle with a reduced quality and satisfaction of life as a result of unremitting negative symptomology, as well as healthcare systems due to the lack of discovery of efficacious therapeutic treatment for negative symptoms (Sicras-Mainar et al., 2014, Sarkar et al., 2015). Overall, individuals that display persistent negative symptoms may experience severe and enduring adverse outcomes that compromise occupational or academic performance, contribute to huge reductions in quality of life, and may even lead to the diagnosis of 'deficit syndrome' SCZ (Eack and Newhill, 2007, Foussias and Remington, 2010).

The pathological changes that underpin the presentation and severity of negative symptoms remain elusive, though large bodies of evidence point to specific immune cell dysfunction within the brain and CNS. Research suggests these changes are a consequence of early life stress/trauma or infection that cause later exacerbations in immune responses, which seem to be dominant factors in negative symptom emergence in SCZ. Furthermore, the weight of evidence suggests that immune-inflammatory dysfunction is the driving factor for the emergence of SCZ in general, whilst also predisposing SCZ patients to severe negative symptoms through a shared exacerbated inflammatory cascade (Meyer et al., 2011, Müller, 2018). This is highlighted through the characteristic increases in immune activation, proinflammatory cytokine production, and oxidative damage that is present both during initial psychosis and established SCZ (Miller et al., 2011, Réus et al., 2015, Bloomfield et al., 2016). Inflammatory dysfunction is far reaching, and is known to activate downstream

signalling cascades that contribute to depressive-like behaviours, cognitive deficits, brain volume and connectivity alterations (Howes and McCutcheon, 2017). Therefore, a greater understanding of the specific mechanisms and how this relates to the presentation of negative symptomology in FEP/SCZ is critical in determining more effective therapeutic treatment for patients, which is currently lacking.

2.0 Mechanisms of Disease Manifestation

2.1 Genetic Predisposition to SCZ Development: Copy Number Variants & Single Nucleotide Polymorphisms

SCZ pathology undoubtedly possesses an extremely heritable genetic component, with over 100 genetic variants recognised as 'risk factors' for SCZ. This consists of common and rare allele variants that have varying penetrance, and may accumulate to predispose individuals to the development of SCZ (Ripke et al., 2014). The most common genetic variations that are present in SCZ include 'Copy-Number Variants' (CNVs), and 'Single Nucleotide Polymorphisms' (SNPs). CNVs are alterations specific genes that include the deletion or duplication of various sections of DNA that are associated with increased risk of SCZ (Bassett et al., 2010). SNPs represent a single alteration in one of the 'building blocks' of DNA that can contribute to the emergence of SCZ. Overall, These can cause alterations in DNA that contribute to maldevelopment of various receptors, enzymes, neurotransmitter channels, and cell constituents that dramatically increase the predisposition to disease, in which over 8000 SNPs are identified as potential contributors (Harrison, 2015).

Interestingly, SNPs can cause alterations in immune functioning. Multiple genome-wide association studies reported polymorphisms of Major Histocompatibility Complex (MHC)

genes that are heavily related to immune function and development, and are associated with an increased risk of SCZ development. Aberrant expression of the MHC genes within the brain may lead to excessive synaptic pruning, as well as alterations in synaptic function and plasticity that is important in SCZ manifestation (Mokhtari and Lachman, 2016). Further aberrations are reported in immune-related genes that appear to be significantly upregulated, which increases the risk of immunological activation in SCZ as a consequence of genetic inheritance, and may be later exacerbated by interaction with environmental factors (Ripke et al., 2014). For example, genetic studies have reported significant upregulations of a whole host of immune related genes within the hippocampus of SCZ patients when compared to control patients, including CHI3L1, which is known to be upregulated in response to stress and inflammation, therefore may be indicative of a central proinflammatory state in SCZ (Hwang et al., 2013).

2.2 Environmental Stressors: Optimal Functioning & Dysfunction in SCZ

Given that genetics-based research has generally suggested SCZ genetic heritability to be at approximately 80%, attention has turned to the possible role that environmental factors can play in the contribution to the development of SCZ pathology (Hilker et al., 2018). Large bodies of evidence suggest that exposure to environmental stressors that may occur as early as prenatally, throughout childhood, and into adolescence may interact with genetic risk factors and accelerate deterioration into disease (Brown, 2011). Multiple mechanisms have been suggested as important in SCZ emergence, with the main focal point surrounding oxidative stress, neuroinflammation, and alteration of signalling cascades in the brain and CNS.

2.2.1 Redox Balance & The Role of Glutathione

Although the early pathology of SCZ development is not fully elucidated, evidence points to the contribution of excessive amounts of oxidative stress in the brain that can cause immune dysregulation. This can lead to subsequent neuroinflammation and further free radical production and oxidative stress that is important in the emergence of SCZ pathology, through a potential 'reciprocal relationship' whereby one may activate the other (Barron et al., 2017). The brain is particularly susceptible to oxidative stress and tissue damage due to its' high oxygen utilisation (20% total oxygen, when accounting for 2% bodyweight). In metabolism, reactive oxygen species (ROS) are produced after stress as well as during normal metabolic processes, for example oxidative phosphorylation, and are subsequently quenched by antioxidants (Pizzino et al., 2017). Moderate amounts of ROS that are produced during metabolism are considered as crucial for proper neuronal development and represent elements of biological signalling pathways that underpin various physiological processes, for example ROS involvement as messenger molecules through interaction with protein kinases for synaptic plasticity and glutamate signalling (Knapp and Klann, 2002). However, excessive production of free radicals may be damaging towards cells and must be quenched by antioxidants in order to prevent oxidative damage that may compromise brain functioning (Salim, 2017). Glutathione (GSH) is considered a vital antioxidant enzyme that functions to prevent excessive ROS production and oxidative stress. The glutathione cycle is displayed in Figure 2.

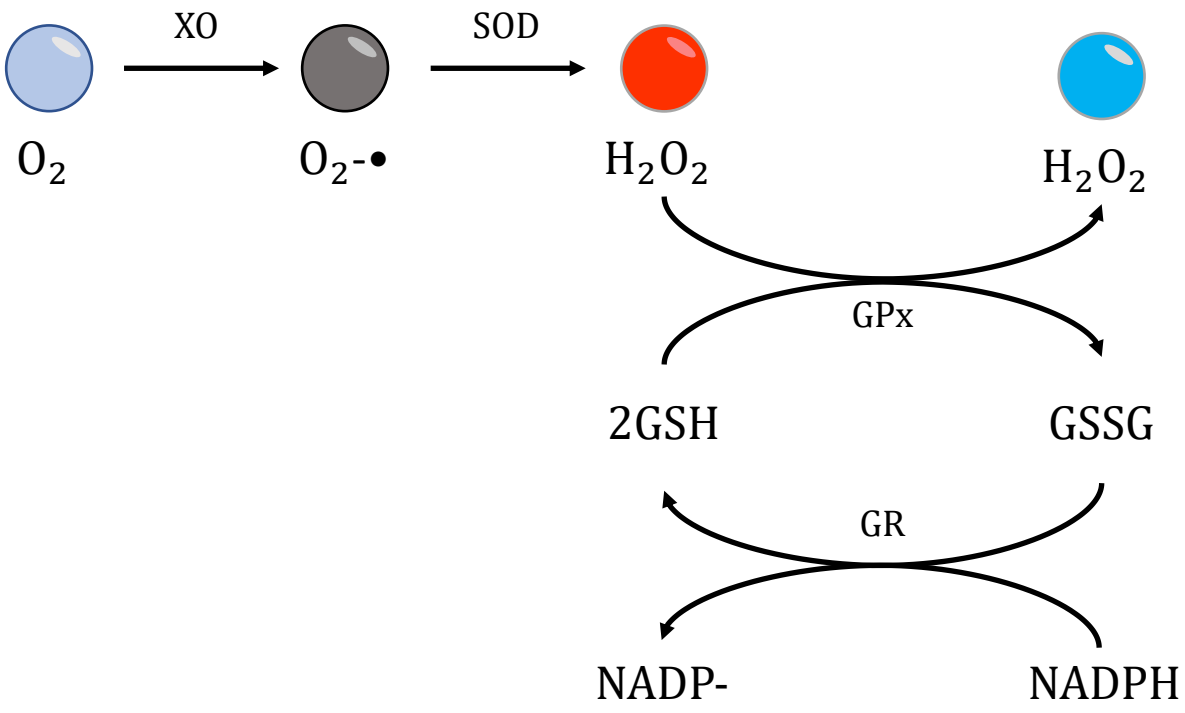


Figure 2. A schematic displaying the conversion of superoxide (O2•−) into hydrogen peroxide (H_2O_2) and water (H_2O) by GSH to prevent tissue damage in the brain. After removal of superoxide, GSH is recycled back into its' original form, replenishing antioxidant defences in the brain. XO, Xanthine Oxidase; SOD, Superoxide Dismutase; GPx, Glutathione Peroxidase; GR, Glutathione Reductase; GSH, Glutathione; GSSG, Oxidised Glutathione; NADPH, Nicotinamide Adenine Dinucleotide Phosphate.

Altered redox status caused by depletion of antioxidants such as glutathione (GSH) can lead to excessive ROS generation and oxidative stress, which therefore represents a key biological stimulus that is crucial for the rapid initiation of an efficient immune response in the CNS (Filomeni et al., 2002). In the event of GSH depletion and ROS generation, the immune system offers protection against infection and stress through the upregulation of both proinflammatory and later anti-inflammatory cytokines in order to exacerbate inflammation, facilitate the removal of pathogens, and subsequently downregulate inflammatory markers in an integrated immune response. At physiological concentrations, GSH constitutes one of the main antioxidant defence mechanisms which operates via efficient quenching of free radicals through oxidation of GSH itself in order to keep ROS under control, which protects the brain from damage (Mailloux et al., 2013). In pathological conditions where total antioxidant capacity may be compromised, overwhelming radical production may cause excessive

activation of the immune system. This may provoke an inflammatory response whereby chronic secretion of proinflammatory cytokines can contribute to further pathological production of free radicals and unprecedented neuroinflammation (Koga et al., 2016). Furthermore, the innate immune cells of the brain can also generate superoxide radicals through NADPH oxidase in order to destroy pathogens. Therefore, if insufficiently regulated, this response can contribute to the development of disease pathology, due to dysregulation of redox and oxidative stress mediated reductions in cell survival that is exacerbated by inflammatory mechanisms and neuroinflammation (Bitanihirwe and Woo, 2011, Barron et al., 2017). Indeed, it has been reported that SCZ populations display an impairment with regards to antioxidant defence mechanisms, alongside upregulations in markers of oxidative stress and defective cytokine concentrations, which may further suggest an interplay of these different pathways in disease manifestation (Fraguas et al., 2019).

2.2.2 Optimal Immune Response to Radicals

The immune system of the brain and CNS operates via two distinct subsystems based on their level of specificity in order to combat infection and provide protection against later exposure to pathogens. The first subsystem is termed the innate immune system, which functions to defend against pathogens, stressors, injury, and foreign bodies through rapid generalised responses in order to eradicate pathogens and prevent infection. Conversely, the adaptive immune system is activated more slowly if the infection is insufficiently cleared by the innate defence mechanism. This branch of immune defence can generate antigen specific responses based on a memory function in order clear infection and prevent the presentation of symptoms of disease via large a series of different antibodies (Vivier and Malissen, 2005).

Within the brain, the microglia represent the initial defence mechanism of the innate immune system, accounting for approximately 20% of total brain cells (Harry and Kraft, 2012). These cells develop in the embryonic yolk sac before migration to the brain where they fully integrate into the CNS. During development, the microglia expand and grow into mature cells that are important in regulation of neural circuits, synaptic profiling, and most importantly the immune response to stressors (Nayak et al., 2014). Cell-based models have reported that the microglia regulate synaptic density and functioning in the brain by engulfment and pruning of synapses, which is an important process for effective signal transmission and cerebral connectivity during neurodevelopment (Ji et al., 2013). Contemporary research that has utilised electron microscopy confirmed the role of the microglia in 'sculpting' the brain, as evidence of cellular debris from pre-and postsynaptic neurons have been discovered inside microglial cells, whereby microglia favour engulfment of neuronal synapses activated less frequently (Hong et al., 2016). These studies show that microglial development and function is crucial for normal brain health as these cells are influential in directly regulating regional connectivity within the brain via synaptic connections, as well as possessing a vital role in defence against infection, tissue damage, and insults, through the secretion of various inflammatory factors (Kierdorf and Prinz, 2013).

During health, the microglia remain in a 'quiescent state', and engage in a passive role of surveying their surrounding environment, and interact with receptors on nearby neurons and astrocytes (Michell-Robinson et al., 2015). However, upon encountering stress or infection and the release of free radicals, the microglia undergo a shift in morphology, which is detected primarily by toll-like receptors on the microglial cell surface. The microglia transition to an amoeboid morphology which is characterised by a rounded cell body, with shorter processes. This morphological change allows the efficient migration of microglia to the site of infection, in order for the microglia to undertake immune processes (Zhang et al.,

2018). Injured cells release neurotransmitters that can be detected by receptors on the microglia to initiate migration to infection site, and can cause activation of a crucial immune process characterised by the upregulation of proinflammatory cytokines, in order to combat infection (Réus et al., 2015). Cytokines are small proteins that are a by-product of stress, and function to modulate inflammation, facilitate pathogen removal, and the resolution of infection (Kronfol and Remick, 2000). Under healthy conditions there is an efficient proinflammatory response that combats infection via upregulation of proinflammatory cytokines and regulated ROS production, as well as the microglial phagocytic engulfment of pathogens and resolution of inflammation, before the shift back to a surveillance state in homeostasis (Orihuela et al., 2016).

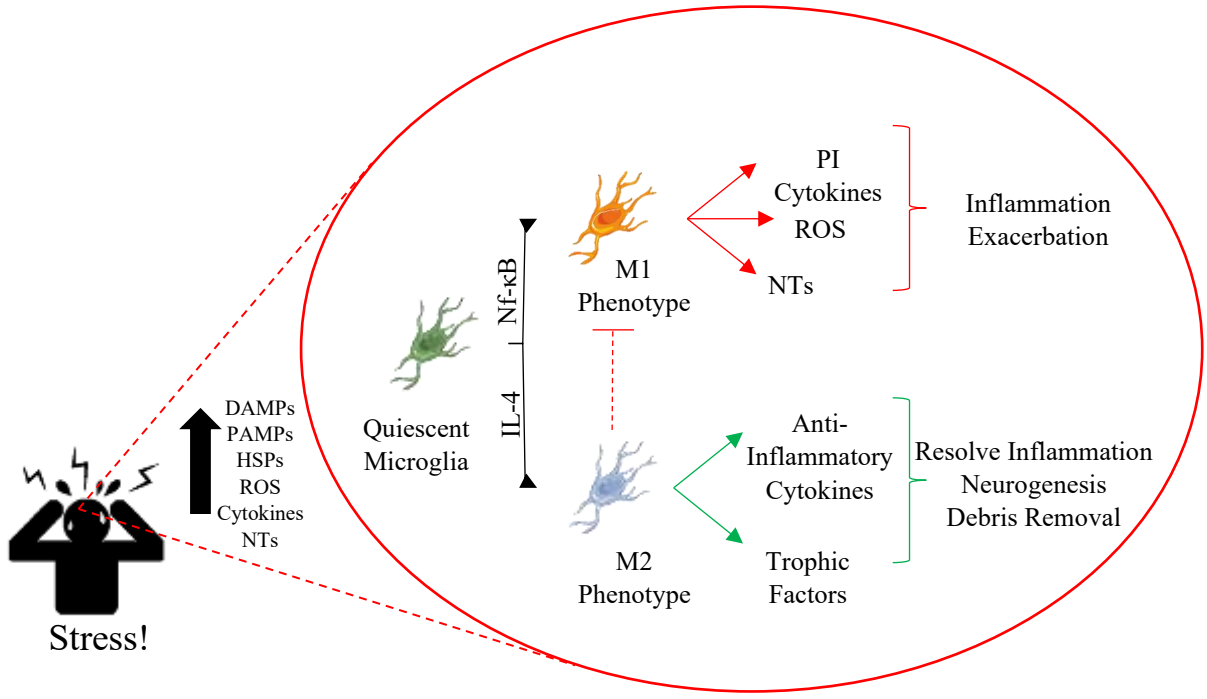


Figure 3. A schematic to display the immune-inflammatory response to stress. Stress causes the release of various neurotransmitters that activate 'classically activated' M1 microglia, which causes an upregulation of inflammation through release of proinflammatory factors. 'Alternatively activated' M2 microglia secrete anti-inflammatory and trophic factor in order to promote survival and damage repair in the brain. DAMPs/PAMPs, Damage/Pathogen Associated Molecular Pattern; IL-4, Interleukin-4; Nf-κB, Nuclear Factor-Kappa B; PI Cytokines, Proinflammatory Cytokines; ROS, reactive oxygen species; NTs, neurotransmitters.

The main proinflammatory cytokines that are upregulated in response to stressors can be categorised based on their associated function. A contemporary review proposed that Tumor Necrosis Factor-alpha (TNF-α), Interleukin-1 (IL-1), IL-6, and IL-8 constitute the initial

exacerbation of inflammation against pathogens. Interferon-Gamma (IFN-γ) and IL-12 promote further inflammation in order to kill intracellular parasites, constituting the T-Helper 1 immune response. On the other hand, IL-4, IL-5, and IL-13 function to compensate for neuroinflammation with anti-inflammatory effects that extinguish neuroinflammation, termed the T-Helper 2 immune response. IL-17 and IL-23 are predominantly proinflammatory and function in order to further exacerbate the inflammatory state and provide a defence mechanism against infection. Finally, IL-10 and Transforming Growth Factor-Beta (TGF-β) dampen the inflammatory response of the aforementioned cytokines, and represent the T-Regulatory response (Momtazmanesh et al., 2019). These cytokines are differentially secreted based on the physiological state within the CNS, and the morphology of the microglia as a consequence of stressors, microenvironment, and disease status. Balance between the Th1, and Th2 inflammatory responses are crucial for an efficient and regulated immune response, and perturbation of this balance can result in chronic neuroinflammation and deterioration into disease (Kim et al., 2004).

Contemporary research shows that the microglia exhibit two distinct phenotypes when engaging in the immune response. Firstly, the microglial 'M1' phenotype emerges in response to an immune challenge via activation of the Nuclear factor kappa B (Nf-κB) pathway. Activation of innate immune receptors causes the phosphorylation of IκB inhibitory sub-units on Nf-κB by specific enzymes (Kopitar-Jerala, 2015). This process allows dissociation and translocation of Nf-κB to the nucleus in order to stimulate secretion of proinflammatory cytokines via the induction of proinflammatory genes (TNF-α, IL-6, IL-1β, etc.), ROS production (nitric oxide, superoxide), as well as secretion of other neurotransmitters (Dresselhaus and Meffert, 2019). This phenotype is amoeboid, proinflammatory, and functions to eliminate infection or pathogens within the brain and CNS. M1 or 'classically activated' microglia appear to replicate the T1 helper cells in the immune

response by aggravating the neuroinflammatory environment through proinflammatory marker secretion (Eggen et al., 2013). This has been suggested to cause alterations in behaviour and symptom emergence that that is present in individuals harbouring an infection, termed ‘sickness behaviour’ (Dantzer et al., 2011).

The second microglial phenotype, ‘M2’ or ‘alternatively activated’ microglia is concerned with resolution of the inflammatory response via expression of IL-4, IL-10, BDNF, COX1 (Réus et al., 2015). Shifting to the M2 phenotype appears later in the immune response, and function ultimately to prevent neuronal damage and promote repair of cerebral tissues within the CNS. This may occur via clearance of cellular debris, and upregulations in the production of anti-inflammatory cytokines and trophic factors (Najjar et al., 2013). Alternatively activated microglia typically replicate the T2 helper response via extinguishing proinflammatory action in order to allow repair of damaged tissue (Cherry et al., 2014). Promotion of the M2 phenotype has various proliferative functions, as it possesses the capacity to stimulate neurogenesis, repair tissue, and also inhibit further M1 activation of microglia via Nf-κB inactivation, in order to dampen the inflammatory state (Cherry et al., 2014, You, 2018). The differential actions of the microglia are displayed in figure 3.

Research to date has thus demonstrated the differential activation of the microglia in an integrated immune response that functions to prevent excess damage within the brain and CNS. This occurs through the simultaneous regulation of M1/M2 activation phenotypes dependant on the specific microenvironment in which the microglia are situated. Dysfunction at the level of the microglia may be key in understanding inflammatory processes that underpin negative symptoms in SCZ.

2.2.3 Oxidative Stress & Immune dysfunction: The 'Vulnerability-Stress-Inflammation' Model of SCZ

Taken together the vital role of antioxidants and the efficient immune responses to stress in maintaining redox balance and preventing excessive damage to the brain, it is interesting to note that large bodies of contemporary research report defects in both antioxidant capacity and immune regulation in the brain that may in part underpin SCZ pathology. There is a substantial amount of evidence of a significantly compromised antioxidant status in SCZ, as several studies have reported downregulations in antioxidant status, including particular reductions in catalase, superoxide dismutase, and the brains' most abundant antioxidant, GSH (Do et al., 2000, Raffa et al., 2011, Flatow et al., 2013). Genetic studies have proposed associations between genetic variations in rate-limiting glutathione synthesis enzymes, for example glutathione cysteine ligase, and severe oxidative stress (Gysin et al., 2007). This is suggestive of a potential mechanism for depleted GSH concentration that is responsible for increased oxidative damage in SCZ (Maas et al., 2017). Polymorphisms in genes that are responsible for the synthesis of antioxidants are suggested to contribute to the inability to generate a sufficient antioxidative response mechanism, exposing individuals to high levels of radical mediated damage in the brain (Chowdari et al., 2011). This is because the remaining antioxidants that are present in the brain and CNS are unable to sufficiently quench radical action, therefore overwhelming free radical production as a result of environmental stress can cause the oxidation of different cellular components and organelles (Wu et al., 2013).

Evidence of impaired antioxidant defences and perturbed redox status in SCZ is furthered by studies that have investigated oxidative stress biomarkers, which reported increased Thiobarbituric Acid Reactive Substances (TBARS), F2-Isoprostanes, protein carbonylation,

and DNA damage in plasma, and cerebrospinal fluid of the SCZ population, which is indicative of radical action having taken place (Zhang et al., 2010, Copoglu et al., 2015, Lee et al., 2016). Oxidative stress is immensely damaging towards cells, as radical action can cause the loss of sulfhydryl groups, and the subsequent modification of proteins may underpin dysfunctional processes. For example, ROS production that cannot be quenched by an overwhelmed antioxidant system, may cause mitochondrial dysfunction through oxidation of essential proteins and enzymes that are vital for oxidative phosphorylation (Rajasekaran et al., 2015). This is an important defect that may lead to unprecedented increases in mitochondrial ROS production and degeneration of cells that may accelerate the development SCZ pathology (Bitanihirwe and Woo, 2011). Moreover, elevated ROS production by mitochondria may induce DNA damage and alter apoptotic processes of neuronal cells in the brain, which has also been implicated in SCZ (Ershova et al., 2017). Contemporary research has suggested that a perturbed antioxidant status is present as early as the FEP population (pre-SCZ) and implies that abnormalities in antioxidant availability and functioning may be an important factor in the genesis and development of established SCZ pathology (Langbein et al., 2018, Fraguas et al., 2019).

Therefore, given that there is evidence of a reduced antioxidant capacity that is present in individuals who go on to develop SCZ, stress at any stage of development through to adolescence could contribute to excessive oxidative damage and perturbation of redox status. Perhaps one of the most important consequences of oxidative stress is that ROS can cause the excessive upregulation of transcription factors that promote a proinflammatory response, namely Nf- κB, and therefore lead to unprecedented increases in proinflammatory cytokine production (Barron et al., 2017). Song et al. (2009) reported a significant increase in Nf-κB activation in serum of a SCZ cohort when compared to a control group, which correlated to an increase in mRNA expression of proinflammatory cytokines IL-1β and TNF-α, providing

support for the overactivation of inflammatory signalling cascades and secretion of cytokines, which may be a consequence of well documented free radical production in SCZ (Song et al., 2009). Further studies conducted in a post-mortem SCZ population, investigating inflammatory markers within the prefrontal cortex also reported significantly increased levels of mRNA, kinases, and cytokine transcripts associated with the Nf-κB signalling cascade, and suggested that this may be indicative of long term elevations in immune signalling within the brain and CNS of the SCZ population (Volk et al., 2019).

Taken together, excessive Nf-κB activation and subsequent cytokine secretion is suggested to cause chronic neuroinflammation that may contribute to the destruction and alteration of vital brain areas that underpins SCZ pathology (Watanabe et al., 2010, Bitanihirwe and Woo, 2011). This appears to be the case, as multiple meta-analyses conducted in the peripheral blood and cerebrospinal fluid of SCZ patients highlight that alongside a compromised antioxidant status and increased markers of oxidative stress, SCZ patients exhibit chronically increased proinflammatory cytokine concentration, including elevations in IL-1β, IL-6, IL-12, IFN-γ, TNF-α (Miller et al., 2011, Upthegrove et al., 2014, Goldsmith et al., 2016). In the review conducted by Miller et al. (2011), the authors suggested that IL-1β, IL-6 and TGF-β were significantly upregulated peripherally in first episode and acute relapse patients, and therefore described these cytokines as 'state markers' that are upregulated amid a psychotic episode and may normalise following antipsychotic therapy. Whereas IFN-γ, TNF-α, and soluble IL-2 receptor remained upregulated across FEP, acute relapse, and stable medicated patients and were described as 'trait' markers that appear to be unaffected by therapeutic medication (Miller et al., 2011). Interestingly, one systematic review also suggested that IL-1β, IL-6, sIL-2r, and TNF-α are aberrantly secreted as early as FEP, which supports the hypothesis of a dysregulated cytokine production as a cause, as well as a consequence of SCZ pathology development (Upthegrove et al., 2014).

The prevailing hypothesis that attempts to tie together the events that lead to chronic neuroinflammation and oxidative stress that is characteristic of SCZ, is the 'vulnerability-stress-inflammation' model proposed by Norbert Müller. This model incorporates genetic predispositions, environmental stress, and immune dysfunction as a set of conditions that facilitate deterioration into disease. This model operates on the basis of genetic susceptibility, which may firstly increase the risk of maladaptive responses to environmental stressors through CNVs and SNPs. This may translate to alterations in immune responses to stress which operate on a 'two-hit' basis to cause increased inflammation and SCZ risk (Müller, 2018).

The vulnerability-stress-inflammation model suggests that in the presence of an increased genetic risk, environmental stressors can interact with developing brain cells, particularly the microglia, to exacerbate dysfunction and promote chronic neuroinflammation. This hypothesis proposes that early life environmental stressors, described as a 'first hit' that may occur as early as prenatally, can contribute to the 'sensitisation' and priming of the microglia towards a proinflammatory (M1) phenotype in the CNS during development (Müller, 2018). This is suggested to occur as a consequence of genetic modifications that expose individuals to a maladaptive response to environmental stressors. This can lead to defective upregulations of ROS that cannot be sufficiently quenched, as well production of as DAMPs/PAMPs, ATP, and heat shock proteins, which interact with a multitude of receptors on the microglia to cause chronic activation and sensitisation of these cells (Monji et al., 2013). Upon 'sensitisation', the microglia become increasingly receptive to stress, causing a reduction in the threshold required to elicit an amplified inflammatory response. This means that weaker, less stressful stimuli have the capability to cause exaggerated proinflammatory responses that may contribute to neuroinflammation that is present in SCZ, due to the increase in vulnerability, and subsequent exposure to stress (Sparkman and Johnson, 2008). Therefore,

'second hits' of stressful stimuli that may occur at any point throughout childhood and into adolescence can cause exacerbated immune action, oxidative stress, and further proinflammatory cytokine release that may underpin long-term neuroinflammation and SCZ pathology (Müller, 2018).

Overall, this facilitates an environment of exacerbated cytokine release and neuroinflammation which hypothesised to cause the alteration in multiple downstream cascades that are associated with the emergence of SCZ symptomology, as well as the overactivation of the microglia to the M1 phenotype that can engage in further neurodegenerative processes in SCZ (Howes and McCutcheon, 2017). This hypothesis is illustrated in figure 4.

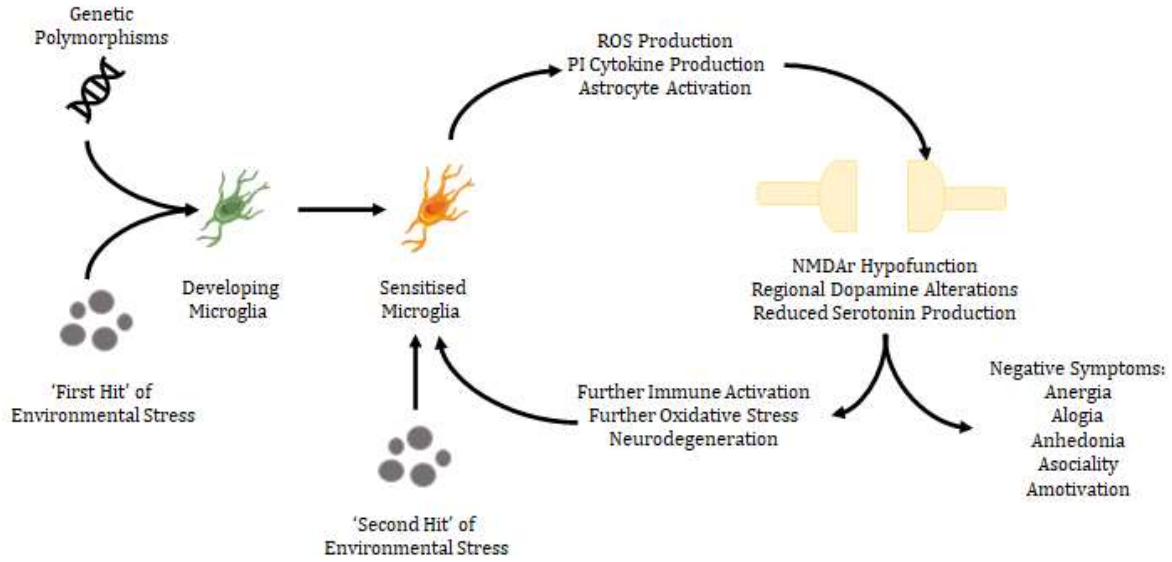


Figure 4. A diagram displaying the 'Vulnerability-stress-inflammation' model, adapted from Muller (2018). Genetic polymorphisms and an early 'hit' of environmental stress causes the sensitisation & priming of microglia to a proinflammatory phenotype during development. Consequently, later environmental 'hits' that are distributed temporally throughout adolescence can cause dysregulated ROS and PI cytokine release, and astrocyte activation. This causes the alteration of downstream cascades that ultimately cause the emergence of SCZ pathology and symptomatology. The proinflammatory environment of the brain and CNS causes further immune activation in a futile positive feedback style system. ROS, Reactive Oxygen Species; PI Cytokine, Proinflammatory Cytokine; KYNA, Kynurenic Acid

Multiple rodent based SCZ models suggested that early life stress and infection can cause increased priming and activation of microglia in offspring that is associated with abnormal cytokine signalling and schizophrenia-like behaviours (Juckel et al., 2011, Zhu et al., 2014). Furthermore, it has been reported in human studies that viral infections of the CNS during childhood may contribute to an increased risk of SCZ development, which could be a consequence of infection-induced sensitisation of immune cells during the 'two hit' process that facilitates defective inflammatory processes (Khandaker et al., 2012). This appears to be the case, as a meta-analysis by Wang & Miller (2018) reported chronically upregulated IL-1β, IL-6 and IL-8 concentrations in the cerebrospinal fluid of SCZ patients. This is the closest proxy to direct measurements of the brain microenvironment, therefore providing further support for central neuroinflammation in SCZ pathology.

Advancements in contemporary research methods have enabled the direct investigation of inflammation and real-time manifestation of schizophrenia *in vivo*. Exacerbated central immune activation appears to be present in SCZ, as evidence from Positron Emission Tomography (PET) scans conducted *in vivo* suggested that the microglia are hyperactivated in the grey matter of the brain, displayed by specific increases in 18-kDa translocator protein radioligand binding to activated microglia in patients when compared to healthy controls (Laskaris et al., 2016). Brain scanning studies conducted by Doorduin et al. (2009), reported specific increases in activated microglia in the hippocampus of SCZ patients and suggested that regional microglial activation may relate to specific symptoms and characteristics of disease. Interestingly, neuroimaging studies conducted by Bloomfield et al. (2016), suggested that microglial activation was increased in SCZ patients, but was also present in 'high risk' of psychosis patients, with significant activation reported in the frontal lobe. The degree of activation in 'high risk' patients was positively associated with the severity of symptoms

experienced, suggesting that microglial activation may be linked with the progression of disease and potential transition to psychosis and SCZ.

Taken together the research conducted in rodents, with peripheral and central measures of cytokines and immune activation in humans, an exacerbated immune response that is present in SCZ appears to consist of a large increase in M1 microglial activation, which may promote both chronic central and peripheral neuroinflammation (Nakagawa and Chiba, 2014, Marques et al., 2019). If such an exacerbated immune response occurs persistently in the brain as it is known to in SCZ pathology, this may form part of a vicious positive feedback loop whereby upregulations of proinflammatory cytokines facilitate to further inflammation (Müller, 2018). This hypothesis is consistent with meta analyses that report peripheral cytokine alterations in psychosis and SCZ (Miller et al., 2011, Upthegrove et al., 2014). Indeed, it has been suggested that the increased presence of inflammatory markers in the periphery show similarities with those measured in the cerebrospinal fluid, and further implicates central inflammation in SCZ pathology (Wang and Miller, 2018). This may cause progression of SCZ pathology as the excessive activation of immune cells and chronic production of cytokines can promote excessive apoptosis, neurodegeneration, oxidative stress, synaptic pruning and widespread neuronal loss in various brain regions (Monji et al., 2009, Réus et al., 2015, Goldsmith and Rapaport, 2020). Neuroinflammation and microglial activation has also been suggested to contribute to specific reductions in brain volume in SCZ patients. For example, particular decreases in prefrontal grey matter volume have been reported as negatively correlated to inflammatory markers including IL-6 mRNA (Zhang et al., 2016). This is relevant as grey matter losses in multiple areas including the prefrontal areas, hippocampus and cerebellum have been suggested to possess importance with regards to negative symptom emergence as early as pre-psychosis, and may be mediated by inflammatory processes (McKechanie et al., 2016, Howes and McCutcheon, 2017).

Interestingly, there is evidence to suggest that chronic low-grade inflammation can contribute to deleterious effects within the brain in SCZ, and the alteration in specific downstream pathways that may contribute specifically to negative symptom emergence in SCZ (Miller and Goldsmith, 2019).

3.0 Inflammation as a Central Hub for Negative Symptom Emergence

3.1 Overview

Given the well characterised relationship between perturbed cytokine expression and SCZ development, contemporary research has diverted attention to the prospective contribution of proinflammatory cytokines to specific symptom profiles within SCZ disease pathology. The downstream effects of chronic neuroinflammation in SCZ are widespread and can contribute to the broad range of symptomatology experienced. However, there is evidence to suggest that neuroinflammation may contribute specifically to the emergence of negative symptoms, though this area of research has been largely neglected until recent years. Multiple cytokines have been reported as associated with the severity of negative symptoms as measured by the PANSS and SANS negative symptom scales. One study suggested that proinflammatory cytokines were predictive of the trajectory of negative symptoms independently of depression over a follow-up period of 1 year in a chronic high risk cohort, suggesting importance to the downstream effects of proinflammatory cytokines even in the earliest stages of pathology (Goldsmith et al., 2019). Furthermore, deficit SCZ which is characterised by enduring negative symptoms, appears to retain a distinct proinflammatory profile that is even significantly different from non-deficit SCZ (Goldsmith et al., 2018). This may provide support for a potential cytokine-induced emergence of negative symptoms. However, the full

extent of the effects of inflammation are not known, therefore effective therapeutic treatment is lacking. Contemporary research centres on inflammation as a central hub for the emergence of negative symptoms, through excessive cytokine production that contributes to the perturbation of downstream pathways including serotonergic, glutamatergic, and dopaminergic neurotransmission which may aggregate to cause presentation of negative symptomology.

3.2 Reduced Serotonin Availability

Large bodies of evidence have suggested that increased glial activation and subsequent cytokine production is associated with defective monoamine signalling within the brain, and that these mechanisms are heavily implicated with negative symptom emergence in SCZ. In particular, alterations in serotonergic transmission are concerned, as the depletion of serotonin (5-HT) can cause multiple detrimental downstream effects that may manifest in the presentation of negative symptoms in SCZ. 5-HT is a key neurotransmitter involved in mood, emotion regulation and cognition, and is reported to innervate most regions within the brain through secretion of synaptic 5-HT that binds to 5-HT receptors ($5\text{-}HT_1$-$5\text{-}HT_7$). These receptors govern a variety of functions that ultimately regulate the overall serotonergic transmission within the brain (Abi-Dargham et al., 1997). Therefore, early research suggested that aberrations in concentration, binding, and turnover of 5-HT may contribute to alterations in affective functioning, and have been reported as associated with negative SCZ pathology (Abi-Dargham et al., 1997, Winograd-Gurvich et al., 2006). In particular, alterations in 5-HT were suggested as important in SCZ by neuroimaging studies, which reported regionally altered serotonergic binding in the amygdala in SCZ, whereby lower 5-HT receptor binding was significantly correlated with more severe negative symptoms (Yasuno et al., 2004).

Additionally, early reviews noted a decrease in 5-HT in the cortex of the SCZ sub-populations characterised by negative symptoms (Abi-Dargham et al., 1997). Low synthesis, overall concentration of 5-HT, and receptor binding may be exaggerated by the effects of neuroinflammation, as proinflammatory cytokines have been suggested to increase the activity of serotonin transporters on astrocytes which recycle 5-HT from the synapse to abolish the effects of synaptic 5-HT, therefore diminishing serotonergic neurotransmission (Miller and Raison, 2016).

Interestingly and perhaps most importantly, there is evidence to suggest that proinflammatory cytokines (mainly IL-1β and TNF-α) can cause the overactivation of enzymes that influence 5-HT synthesis and overall concentration. It is suggested that proinflammatory cytokines can cause the excessive activation of the Indoleamine 2,3-Dioxygenase (IDO) which is primarily located in the microglia, and Tryptophan 2,3-Dioxygenase (TDO) in astrocytes, displayed in Figure 5. Activation of these enzymes can cause a diversion in the metabolism of tryptophan, an essential precursor for the synthesis of 5-HT, towards different metabolic end products and limit the availability of tryptophan for 5-HT production. This may impair serotonergic signalling, and has been suggested as important with regards to negative symptoms in SCZ (Barry et al., 2009). In the microglia, IDO causes the metabolism of tryptophan to shift towards production of neurotoxic quinolinic acid (QUIN) which is highly present in depressed individuals (Steiner et al., 2011), and has also been suggested in some SCZ cohorts (Kanchanatawan et al., 2018). Whereas, in astrocytes where TDO is active, tryptophan is alternatively metabolised into kynurenic acid (KYNA), which is a by-product that is well documented in the SCZ population a as potent and upregulated NMDA receptor antagonist (Plitman et al., 2017, Pedraz-Petrozzi et al., 2020), discussed later. Taken together, the alternative metabolism of tryptophan away from the synthesis of 5-HT may contribute to a reduction in the overall concentrations of extracellular 5-HT that is characteristic of

depressed individuals, and may reflect the reduction in serotonergic signalling that is suggested as relevant to negative symptoms in SCZ (Winograd-Gurvich et al., 2006, Mitra et al., 2016). However, the limited efficacy of selective serotonin reuptake inhibitors (SSRI's) with regards alleviating negative symptoms, make definitive conclusions about the direct effect of low serotonin difficult to come by. SSRI's function to prevent 5-HT reuptake from the synapse, therefore the absence of any effect of increasing synaptic 5-HT on negative symptoms may suggest that low serotonin may not be the direct cause of negative symptoms in SCZ.

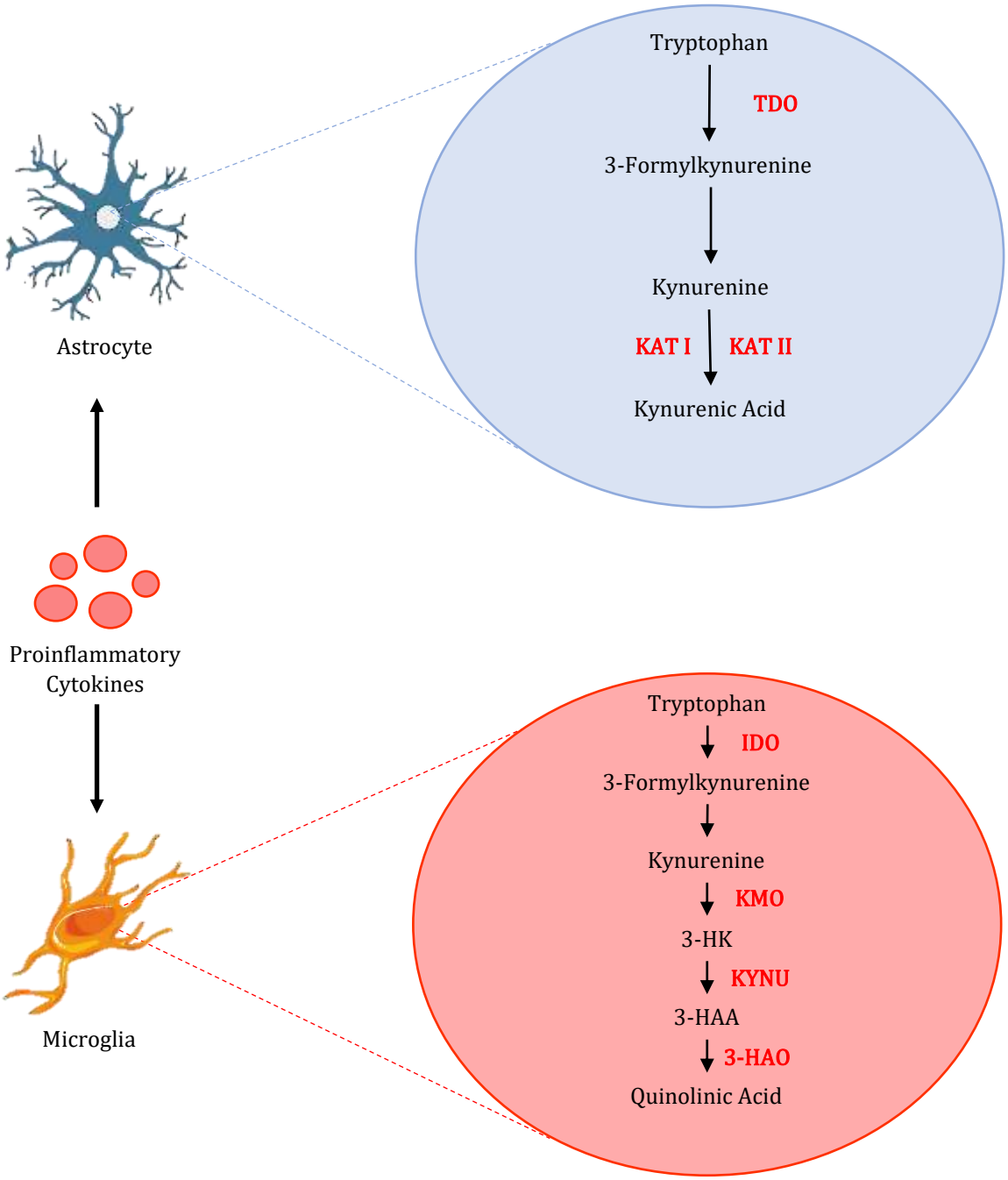


Figure 5. A diagram displaying the alternative metabolism of tryptophan as a consequence of chronic inflammation. In astrocytes, Inflammation causes the activation of TDO, and tryptophan is converted to 3-formylkynurenine and kynurenine. KAT I/KAT II then convert kynurenine into Kynurenic Acid, an endogenous NMDA receptor antagonist. In microglia, inflammation causes the activation of IDO and conversion of tryptophan to 3-formylkynurenine and kynurenine. Kynurenine is metabolised by KMO into 3-HK, and further metabolised into 3-HAA by KYNU. Finally, 3-HAO metabolises 3-HAA into Quinolinic acid, a neurotoxic end-product. TDO, Tryptophan 2,3-Dioxygenase; KATI/II, Kynurenine Aminotransferase; IDO, Indoleamine 2,3-Dioxygenase; KMO, Kynurenine 3-Monooxygenase; KYNU, Kynureninase; 3-HAO, 3-Hydroxyanthranilic Acid Oxygenase. Created in Biorender.com.

Inflammation induced alterations in serotonergic signalling may however, possess potent indirect effects with regards to the emergence of negative symptoms. Within the microglia,

IDO can influence the alternate metabolism of tryptophan towards 3-hydroxykynurenine (3-HK) and QUIN production. Both 3-HK and QUIN are neurotoxic and can contribute to further ROS generation, stimulate further proinflammatory cytokine secretion, and the destruction of cellular components in the CNS (Dantzer et al., 2011). One recent study conducted by Kanchanatawan et al. (2018), reported significantly elevated QUIN concentrations in a deficit SCZ cohort when compared to non-deficit and control, and even suggested that QUIN was predictive of specific items of the SANS questionnaire. The authors suggested that increases in proinflammatory cytokines may facilitate neurotoxic increases in QUIN that may contribute to negative symptoms through further proinflammatory effects, ROS production, glutamatergic dysfunction, and defective mitochondrial function within the brain and CNS (Kanchanatawan et al., 2018). However, the majority of evidence surrounding the cytokine-mediated depletion in tryptophan suggest that the diverging pathway that concludes with QUIN production is more relevant to depressive disorders, as increased QUIN concentration has been reported more frequently in patient and preclinical models of depression (Steiner et al., 2012, Iaccarino et al., 2013), and direct evidence in SCZ is somewhat lacking. Similarly, elevated QUIN as a specific characteristic of depression has also shown on a larger scale via systematic review (Ogyu et al., 2018). Whereas, the majority of patients with SCZ displaying prominent negative symptoms appear to exhibit elevated levels of the second branch of tryptophan metabolism to kynurenic acid, which is highly implicated with alterations in glutamatergic neurotransmission (Müller and Schwarz, 2006), discussed later.

Heterogeneity in SCZ as a disease, as well as duration of illness and medication, may represent dominant confounding factors regarding the variations seen in levels of QUIN and kynurenic acid in disease context. Additionally, regional differences and measurement location in QUIN and kynurenic acid across the brain may be a reason as to why some studies

report increases in these metabolites, where others might report reductions. Overall, despite the prominent increases in neurotoxic QUIN that have been reported previously in depression, increased activity of this branch of the tryptophan depletion pathway has not been convincingly replicated in the context of SCZ. However, this area of research has largely been neglected in SCZ research and QUIN appears to be an understudied metabolite in this context, therefore further elucidation may provide a better understanding of the mechanisms that underpin dysregulated serotonergic transmission surrounding negative symptoms in SCZ.

In recent years, the serotonergic hypothesis in isolation appears to have lost support as a direct cause of negative symptoms in psychosis and eventual SCZ, and the apparent lack of a direct therapeutic effect subsequent to treatment with SSRI'S further suggest that 5-HT concentrations itself may not be critical for negative symptomology (Sepehry et al., 2007). Although, characteristics of dysfunction that are present in this signalling pathway may prove integral to the manifestation of disease, for example dysregulation of the kynurenines. Contemporary evidence suggests that the inflammation induced alternate metabolism of tryptophan that underpins serotonergic dysfunction in SCZ has enormous implications with other signalling mechanisms in the brain that may interact to bring about negative symptoms in SCZ, namely the glutamatergic and dopaminergic system.

3.3 Kynurenic Acid & Glutamate Dysfunction

Perhaps the most important downstream pathway that is affected by neuroinflammation, that is suggested as important in the emergence of negative symptoms is the glutamatergic pathway and the associated glutamate receptors. Excitatory transmission in the brain is predominantly glutamatergic, as these neurons account for up to 80% of total brain metabolic

activity, and blockade of this transmission may lead to affective flattening and negative symptom emergence (Carlsson et al., 2001, Rothman et al., 2003). The NMDA receptor is an ionotropic glutamate receptor that is crucial in promoting glutamatergic neurotransmission and modulation of neuronal excitation throughout the brain, as glutamate is released presynaptically before subsequent uptake by this receptor (Newcomer et al., 2000). NMDA receptors possess a high channel conductance, multiple subunits that permit the binding of glutamate, and are distributed heavily throughout multiple regions of the brain (Hansen et al., 2018). At a physiological level, concentrations of synaptic glutamate are low and tightly regulated, and secretion of glutamate allows for the binding to subunits on NMDA receptors and signal transmission between neurons that is important for brain functioning, learning, memory, plasticity, long term potentiation, and cognition (Riedel et al., 2003). Therefore, defects in glutamate concentrations and aberrations in glutamatergic signalling may have detrimental consequences that lead to the development of disease pathology. The NMDA receptor is located between primary and secondary glutamatergic cortical neurons, and has been proposed as the fundamental deficit effecting both glutamatergic and GABAergic signalling in psychosis and SCZ (Kahn and Sommer, 2015).

The original premise of the glutamate hypofunction hypothesis was formulated on the basis of unrelenting negative symptoms in SCZ even in the presence of antipsychotic treatment targeting the D2 dopamine receptors, which led to speculation that negative symptomatology may be a consequence of different cellular cascades (Coyle, 1996). Extensive research conducted using acute phencyclidine and ketamine administration (known NMDA receptor antagonists) displayed that antagonism of the glutamatergic NMDA receptor was able to induce SCZ-like psychosis symptoms in healthy participants and rodents, including prominent negative symptoms (blunted affect and withdrawal). Therefore, this supported the notion of a glutamatergic NMDA receptor dysfunction as important in the presentation of

negative symptomatology (Krystal et al., 1994, Neill et al., 2014). Acute ketamine administration may more accurately represent the FEP phase of disease, as this has been reported to replicate excessive increases in glutamate production that are also displayed in the psychosis population via Magnetic Resonance Spectroscopy (MRS) scanning studies (Rowland et al., 2005, Egerton et al., 2018, Shakory et al., 2018). One MRS study in particular suggested that glutamate levels in the anterior cingulate cortex were significantly upregulated in a FEP cohort, and were positively associated with increased severity of negative symptoms (Egerton et al., 2012). Whereas, chronic NMDA antagonism by ketamine may represent more long term deficits in glutamatergic signalling and blockade in neuroexcitatory transmission, therefore simulating negative symptoms and cognitive defects exhibited in more established SCZ pathology (Moghaddam and Javitt, 2012, Howes et al., 2015).

On a mechanistic level, neuroinflammation in SCZ may contribute to glutamate hypofunction and negative symptom presentation via the cytokine induced changes in the serotonergic system. The updated glutamate hypothesis suggests that the alternative metabolism of tryptophan subsequent to potent inflammatory activity can lead to the production of KYNA, catalysed by TDO and astrocyte specific enzyme kynurenine aminotransferase (Erhardt et al., 2017), as illustrated in figure 5. This metabolite is suggested as important in SCZ cohorts, as KYNA is identified as the only known endogenous antagonist of NMDA receptors (Stone, 1993). Therefore, excessive kynurenic acid may account for the antagonistic effects induced by ketamine administration that are known to replicate negative symptoms in SCZ. Indeed, central KYNA concentration has been identified as significantly upregulated in SCZ by meta-analysis (Plitman et al., 2017), and has also been positively associated with upregulations in multiple proinflammatory cytokines including IL-6 and TNF-α, suggesting a potential relationship between inflammation and KYNA production (Pedraz-Petrozzi et al., 2020).

Additionally, one group suggested that kynurenic acid was significantly increased to a greater extent in a subset of SCZ patients with a 'high inflammatory profile', when compared to patients without a high inflammatory profile, therefore reaffirming the link of proinflammatory cytokines as a causative factor of kynurenic acid accumulation (Kindler et al., 2019). At high concentrations, kynurenic acid can exert antagonistic action on the NMDA receptor that may cause increased presynaptic glutamate release, alongside a dampening of postsynaptic excitatory glutamatergic neurotransmission and glutamate uptake that can negatively affect brain plasticity, and may lead to the development of negative symptoms (Schwartz et al., 2012).

An important aspect of the glutamate hypothesis is that cytokine-induced kynurenic acid that is generated to a higher extent in psychosis and SCZ, can exert antagonistic effects on gamma-aminobutyric acid (GABA)-ergic inhibitory interneurons within specific brain circuits. Excessive antagonistic action at the NMDA receptor site on inhibitory neurons may cause widespread disinhibition of subsequent glutamatergic pyramidal neurons and excessive release of presynaptic glutamate into the synaptic cleft in multiple areas of the brain (Olney and Farber, 1995, Moghaddam and Javitt, 2012). This is crucial as persistent glutamate toxicity may lead to neurotoxic damage and cell death in prefrontal areas that cause the presentation of negative symptoms, for example affective flattening and anergia (Deutsch et al., 2001, Schwartz et al., 2012). Further, central glutamate neurotoxicity can lead to the subsequent generation of ROS, downregulation of crucial antioxidants, and therefore expose vital brain regions to unprecedented oxidative stress (Dean et al., 2009, Howes et al., 2015). This may catalyse a vicious loop of inflammation induced damage, as oxidative stress and ROS production can impair uptake of glutamate from the synaptic cleft, which in turn causes further radical generation and neurotoxicity (Kumar et al., 2018). Cytokines have also been suggested to directly obstruct glutamate recycling and promote glutamate release via

interaction with glutamate transporters on astrocytes , further hindering clearance and contributing to neurotoxicity (Goldsmith and Rapaport, 2020).

Indeed, excess glutamate and its' metabolites have been previously associated with alterations in grey matter volume in various brain regions in the SCZ population. This may be indicative of excitotoxic cell death in specific brain regions including the amygdala and hippocampus, that could be important in negative symptom emergence (Plitman et al., 2014, McKechanie et al., 2016). Alternatively, in more established SCZ, some studies reported decreased concentrations of glutamate that may be representative of long term antagonism and 'hypofrontality', which refers to the the consistent downregulation in activation of frontal brain regions that has been associated with negative symptoms (Gruber et al., 2014). This evidence suggests that alterations in glutamatergic neurotransmission as a consequence of inflammation are a potential contributing factor to the emergence of negative symptoms in SCZ. The general concepts of the glutamate hypothesis are illustrated in Figure 6.

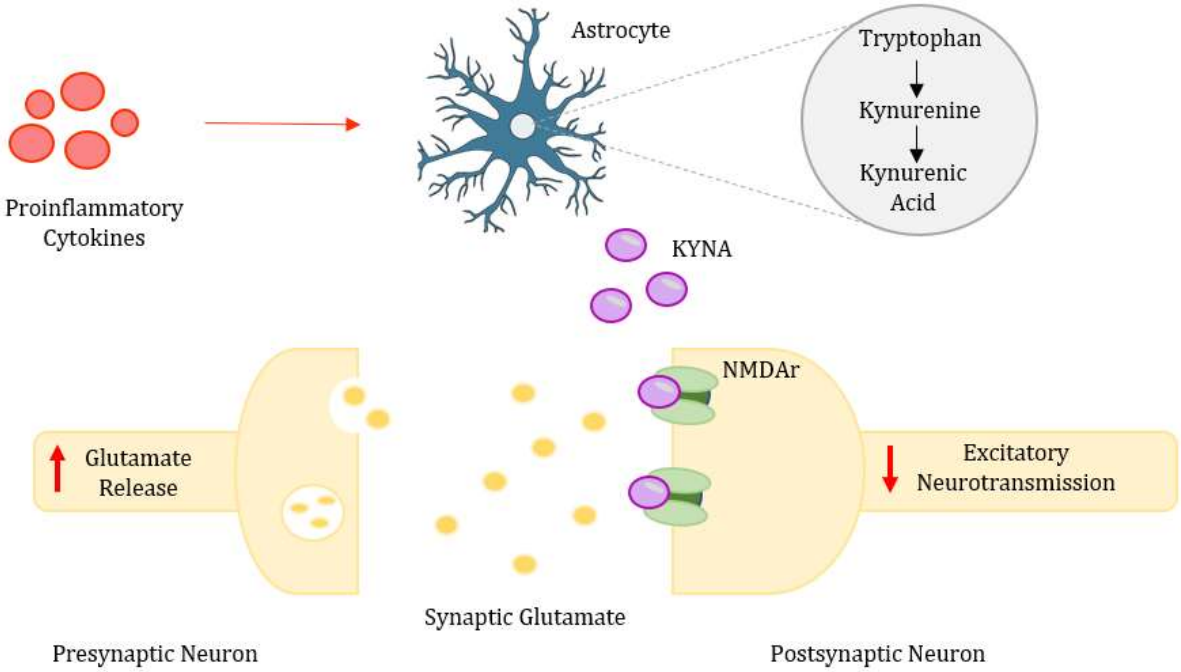


Figure 6. A diagram to display the glutamate hypofunction hypothesis in SCZ. Inflammation can lead to the alternate metabolism of tryptophan to KYNA within the astrocyte. KYNA is released into the synaptic cleft where it can cause the antagonism of the NMDAr, which leads to decreases in excitatory glutamatergic transmission that may contribute to

negative symptom emergence. Consequently, KYNA induced disinhibition of GABA interneurons can cause increased activation and glutamate release from presynaptic glutamatergic neurons into the synapse, which leads to excitotoxicity induced cell death and ROS production as this glutamate is not sufficiently removed. KYNA, kynurenic acid; NMDAr, N-Methyl-D-Aspartate receptor; GABA, Gamma Aminobutyric Acid. Created in Biorender.com.

Crucially, dysfunction of glutamatergic signalling and associated neuroinflammation in brain areas concerned with affective regulation, and may lead to motivational deficits that comprise the negative symptom construct of motivational and consummatory anhedonia (Goldsmith and Rapaport, 2020). This highlights how inflammatory markers may modify and exacerbate the release of glutamate whilst also hindering its' reuptake and signalling, which may contribute to the worsening of psychotic, and particularly negative symptoms.

3.4 Regional Dopamine Alterations

Dopamine is a crucial neurotransmitter responsible for a multitude of different executive functions including motor control, arousal, cognition and motivation. The dopaminergic system operates via nigro-striatal and mesolimbic projections into different brain regions, where dopamine is secreted and subsequently binds postsynaptically via five dopamine receptors (Jaber et al., 1996). Perturbations in dopaminergic transmission have been well characterised in the context of SCZ and represent one of the initial hypotheses of the aetiology of SCZ. Early evidence suggested an increase in central dopamine as well as its' receptors, and this has since been confirmed by more contemporary data that suggests hyperdopaminergia and excessive D2 receptor availability and activation in multiple brain regions. Excessive dopamine concentration is suggested to underpin the development of positive psychotic symptoms including hallucinations, delusions, and disorganisation of speech (Mackay et al., 1982, Howes et al., 2015).However, more contemporary research suggests that dopamine alterations are in fact regional, with hyperactivation in striatal

regions, and deficits in transmission in frontal regions (Vidal and Pacheco, 2020). A popular model for the replication of SCZ pathology was the use of amphetamine as this drug was able to successfully induce psychotic symptoms of impaired cognitive ability and reduced attention by inducing changes in dopamine secretion and function, therefore further implicating this neurotransmitter in the development of disease. (Featherstone et al., 2007).

Dysfunctional secretion of dopamine may occur in SCZ due to hyperactivity of tyrosine hydroxylase in the substantia nigra, which is the rate limiting enzyme for dopamine production and is suggested as important in aberrant dopaminergic signalling that contributes to positive symptoms (Perez-Costas et al., 2012). This hypothesis is derived from research utilising psychostimulant drugs, which were able to amplify striatal dopaminergic signalling to cause psychotic symptoms that relay closely to SCZ symptomatology (Laviolette, 2007). Presynaptic striatal hyperdopaminergia and excessive dopamine release are the most well characterised features of acute psychosis and SCZ, and are the main targets of antipsychotic medication in the interest of minimising positive symptoms through antagonism of the D2 receptor (Howes and Kapur, 2009, Perez-Costas et al., 2012). This is because excessive dopaminergic signalling and receptor binding causes the brain to ascribe importance to stimuli that would usually be discarded, and is described as a potential cause of hallucinatory and delusional symptomology (McCutcheon et al., 2019).

In terms of negative symptomology, regional dopaminergic dysfunction may in fact play a role. Neuroimaging research has suggested that as SCZ patients display impaired activation and transmission of dopamine within the striatal and prefrontal areas of the brain, and that altered dopaminergic signalling within these circuits may lead to presentation of anhedonic behaviour and the emergence of typically negative symptomology (Okubo et al., 1997, Laviolette, 2007, Howes and Kapur, 2009). Interestingly, there is some evidence to suggest that dysfunction dopaminergic neurotransmission may be induced as a consequence of

neuroinflammation that is present in SCZ, and has the capacity to contribute to the severity of negative symptomology. Indeed, cytokines have been shown to possess powerful effects with regards to synthesis, release, uptake, and overall transmission of dopamine. Mechanistically, increased proinflammatory cytokine production that is present in psychosis and SCZ has been suggested to exert detrimental effects on dopamine synthesis, through inflammation induced upregulation of free radicals that limit the availability of tetrahydrobiopterin (BH4), an indispensable precursor of both serotonin and dopamine. Therefore, a reduction in BH4 in turn results in a decrease in the synthesis and release of dopamine and its' presence of downstream metabolites, which may contribute the development of negative symptoms including motivational anhedonia and fatigue (Vidal and Pacheco, 2020). Additionally, inflammatory cytokines may reduce the expression of monoamine transporters that are required for the release and reuptake of dopamine, further reducing dopaminergic signalling in cortical areas, which has been associated with deficits in reward circuitry in SCZ (Felger and Miller, 2012, Goldsmith and Rapaport, 2020). Further, animal based studies have suggested that persistent increases in proinflammatory cytokine administration, namely IFN-α, was able to reduce dopaminergic transmission, and that this reduction was relevant to the increase in anhedonic like behaviours that were exhibited (Felger et al., 2013). This concept may somewhat underpin 'treatment-resistance' experienced in SCZ, as antipsychotics primarily target the D2 receptor in order to reduce dopaminergic signalling, therefore patients that display primarily negative symptoms consequent to regional hypodopaminergia may receive no benefit from current antipsychotic medication (Goldsmith and Rapaport, 2020).

Perhaps most interestingly, dopaminergic signalling is suggested to be in part regulated by glutamatergic and serotonergic transmission. This highlights an interplay between the dysfunction of multiple pathways to cause the emergence of negative symptoms, that are all initially offset by neuroinflammation and the presence of excessive cytokine action. Some

studies have reported that increased kynurenic acid that is present in SCZ as a result of serotonergic perturbations, may result in decreased dopamine concentration through alterations in glutamatergic signalling and subsequent KYNA production (Plitman et al., 2017). One prospective mechanism suggests that inflammatory action that causes production of KYNA may lead to NMDA receptor antagonism on GABAergic interneurons, causing disinhibition of the interneuron and subsequently increased glutamatergic neuron firing. Increased glutamatergic transmission may lead to hyperactivation of a second GABA interneuron, which results in excessive inhibitory action of GABAergic neurons on dopaminergic neurons in the mesocortical areas, resulting in hypodopaminergia of frontal regions of the brain associated with affective flattening (Laviolette, 2007, Schwartz et al., 2012). However, more research is needed in order to determine the full extent to which these integrating mechanisms communicate, as well as the chronology of these prospective defects. Furthermore, heterogeneity of psychosis and SCZ pathology means that this hypothesis must be approached with caution as not all patients display similar aberrations in neurotransmitter pathways.

Taken together, advances in contemporary research have provided crucial support for a defective cytokine upregulation in established SCZ, as well as drug naïve FEP patients (Miller et al., 2011, Upthegrove et al., 2014). Furthermore, there is considerable evidence from individual studies to suggest that inflammatory abnormalities may contribute to the emergence of negative symptoms in SCZ. This may occur through alterations of the serotonergic, glutamatergic, and dopaminergic pathways, displayed by the evident perturbation of neurotransmitter signalling and complex downstream crosstalk that ultimately manifest in negative thoughts and behaviour. Thus, it could be hypothesized that the dysregulation of inflammation and exacerbated cytokine production that is a hallmark of SCZ pathology, may represent a central factor that facilitates the emergence of negative

symptoms. Therefore, investigating the relationship between cytokine concentration and negative symptoms in a FEP population that are early in disease stage and completely antipsychotic naïve may provide a more coherent and valid representation of the prospective relationship between inflammation and symptomology, and may direct future therapeutic treatment. However, to our knowledge the potential association between defective cytokine upregulation and severity of negative symptoms has never been assessed via systematic review and meta-analysis. Therefore, the current review set out to gather all relevant studies to determine whether there is an association between inflammatory markers, and the negative symptoms of SCZ.

4.0 Methods

4.1 Rationale

The aim of this study was to provide an overview of the prospective relationship between a pathological alteration in inflammatory cytokine concentrations, and negative symptom severity in SCZ. Our objectives were to: 1) Provide an up-to-date, comprehensive synthesis of the studies that assess the cytokine hypothesis of psychosis and SCZ, given the ever-present advances in scientific methodology and technique, and 2) delineate the potential role that inflammation plays in contributing to negative symptom emergence and severity in FEP. This may thereby expose a panel of cytokines that could be used in the future as potential biomarkers for the stratification of patients, to determine the most appropriate therapeutic treatment. Notably, Patients that display a prominent negative symptomology at initial clinical presentation have been associated with poorer outcomes, reduced quality of life, worse social/professional functioning, and later admission to hospital (Stahl and Buckley,

2007, Piskulic et al., 2012, Rammou et al., 2019). Therefore if a set of biomarkers can be significantly and consistently associated with negative symptoms in FEP/SCZ, this could be implemented as a diagnostic tool in order to stratify 'high risk' patients and prevent transition over to FEP and eventual SCZ, as well as aid in preventing formation of debilitating negative symptoms.

It was decided that this systematic review would investigate changes in the FEP population as biological markers that are present in SCZ have previously been reported as elevated as early as the first episode (Upthegrove et al., 2014). Furthermore, investigating at the first episode diagnosis will minimise the possible confounding effects of disease duration, exposure to antipsychotics, comorbidities, and will further validate any conclusions that are made regarding inflammation and symptomology in SCZ. As previously discussed, FEP is defined by the DSM-5 as two or more positive, negative, or cognitive symptoms occurring continuously, one of which must present as delusions, disorganised speech, or hallucinations (Diagnostic, 2013). FEP was defined in this review using the DSM criteria or equivalent at the time of publishing, alongside the 'operationalised definition' of FEP proposed by Breitborde et al (2009). This definition considers the potential variation in classification of FEP that is present in clinical and research settings, where a patient may be considered FEP if they are generally early in disease diagnosis, or upon presentation to a clinical setting, without consideration of duration of untreated disease or previous admissions. Breitborde (2009) suggested that the definition of FEP should include multiple constructs, and suggested consideration of a duration of illness of below 5 years, with no previous exposure to clinical treatment/contact, and within their 'first episode' of psychosis i.e., not multiple episodes (Breitborde et al., 2009).

4.2 Study Selection

Prospective studies were systematically searched on 24th July, 2020 via the Ovid database system, using MEDLINE, EMBASE, and PsycInfo databases, in accordance with the PRISMA guidelines to ensure high quality reporting of systematic reviews (Moher et al., 2009). The search was conducted using key words which were also adjusted to subject headings/MeSH headings dependent on the database being searched, with the Ovid 'explode' function implemented where appropriate. Keywords that were mapped to subject headings were also separately searched as a keyword in order to collect all potentially relevant studies and negate any human error regarding database study input or keyword mapping, as advised by library technician. An asterisk '*' marks a truncated keyword where any alternative ending of a word is retrieved.

The key word search terms included: ("schizo*" **OR** "psychosis" **OR** "psychoses" **OR** "first episode" **OR** "first psychotic episode" **OR** "FEP" **OR** "psychotic disorder" **OR** "early onset" **OR** "early intervention" **OR** "drug naïve" **OR** "medication naïve") **AND** ("inflammat*" **OR** "cytokin* **OR** "interleukin*" **OR** "tumour necrosis factor" **OR** "TNF-alpha" **OR** "interferon" **OR** "C-reactive protein" **OR** "immune response" **OR** "immune dysregulation" **OR** "cytokine production) **AND** (negative symptom* **OR** "negative syndrome" **OR** "alogia" **OR** "anergia" **OR** "anhedonia" **OR** "avolition" **OR** "apathy" **OR** "asociality" **OR** "affective flattening" **OR** "blunted affect" **OR** "diminished expression" **OR** "social withdrawal" **OR** "lossADJ1interest" **OR** "lackADJ1interest" **OR** "lossADJmotivat* **OR** "lackADJ1motivat*" **OR** "PANSS" **OR** "SANS").

Additional limits were also implemented into each search, limited to: 1) human-based studies, 2) exclude conference abstracts, 3) search limits from 1982 – 2020, in line with the implementation of the SANS questionnaire (1982) and the PANSS questionnaire (1987) for

quantitative assessment of negative symptoms (Andreasen, 1982, Kay et al., 1987). Studies were then retained or discarded based on an agreed inclusion/exclusion criterion.

Inclusion criteria:

1. Published between 1982 – 2020
2. Patients with a diagnosis of FEP or SCZ
3. Within first five years of duration of illness (explicit measurement in years, or deemed within criteria based on text information, as assessed by author)
4. Medication naïve (or a subset with fully stratified data for FEP)
5. Assessed peripheral circulating cytokines (via blood, serum, plasma)
6. Assessed negative symptoms (via PANSS or SANS only)
7. Apparently healthy control group
8. Human-based only

Exclusion criteria:

1. *In vitro* studies
2. Genetic studies
3. Animal based studies
4. Review articles, Posters, Conference Abstracts
5. Studies not measuring peripheral proinflammatory cytokines
6. Studies not measuring negative symptoms by PANSS Negative or SANS
7. Full text not available
8. Not in English

4.3 Data Extraction & Analysis

Data Extraction

Information was extracted by 2 authors (CD & SA) from each study for a '3 phase exclusion' process of studies for full review. In phase 1 (Identification), any duplicates, posters, and conference abstracts were excluded. Phase 2 (Screening) comprised the exclusion of records based on the title and/or abstract, in accordance with the agreed inclusion/exclusion criterion. In phase 3 (Eligibility), articles were assessed at the full text level, in which population demographic data, duration of illness, study type, medication status, cytokine concentration, symptom scale measurement, and outcome measures were extracted in order to determine eligibility in the systematic review. If specific data required to determine inclusion/exclusion was not available in the online version of a study, an attempt was made to contact the author for supplementary data. If sufficient data was still not available, the study was excluded. Studies were also examined for overlapping/duplicate samples or measurements and excluded accordingly. Mean, standard deviation, and sample size data were extracted separately from each study for each proinflammatory cytokine assessed, for both FEP and control groups, and converted to 'pg/ml' where necessary. Some studies split the FEP group into sub-groups based on certain characteristics of interest. Therefore, in order to combine mean, standard deviation, and sample size of the relevant sub-groups into one homogenous FEP group, the formulas proposed by Cochrane Reviews were implemented:

	FEP	CTRL	Formula
Sample Size	N_1	N_2	$N_1 + N_2$
Mean	M_1	M_2	$\frac{N_1M_1 + N_2M_2}{N_1 + N_2}$
Standard Deviation	SD_1	SD_2	$\sqrt{\frac{(N_1 - 1)SD_1^2 + (N_2 - 1)SD_2^2 + \frac{N_1N_2}{N_1 + N_2}(M_1^2 + M_2^2 - 2M_1M_2)}{N_1 + N_2 - 1}}$

Table 1. A table displaying the formulas used to calculate Sample Size, Mean, and Standard Deviation when combining two subgroups into one larger group (Higgins et al., 2019).

Mean and standard deviation values were also extracted for the PANSS Negative or SANS measurements for the patient groups only. Where appropriate, SANS values were converted into PANSS Negative values based on a formula proposed by Van Erp et al. (2014), in order to allow for comparison of the values across a normalised scale. We concede that for any papers converted from SANS to PANSS Negative, some methodological concerns may arise. The values were assessed by different researchers, therefore inter-researcher variability is unknown. Furthermore, it must be acknowledged that the generalised result for PANSS Negative across all studies is for a population that is heterogeneous by nature, therefore accuracy of conversion may be contested. However, the justification for converting the SANS values to PANSS Negative was on the basis that the patients included in the review and analysis have been selected by criteria that defines them as similar on a demographical and biological basis. Furthermore, Van Erp et al, (2014) conclude that the PANSS Negative score and SANS score are highly related, with the highest accuracy across conversions within the SCZ population for any of the scales converted and assessed ((intra-class correlation = .82 (CI, .66 – .91), N = 29), which we believe justifies the use of the formula for analytical purposes where necessary.

SANS – PANSS Negative Conversion Equation:

PANSS Negative = 7.1196 + (0.3362 * SANS [composite] Total Score)
(Van Erp et al., 2014).

Data analysis

Mean, standard deviation, and sample size for each cytokine assessed in 2 or more studies were inputted into Revman 5.0 for subsequent analysis. A 'random effects' model was selected as this statistical methodology considers heterogeneity that may be present between studies, as well as the variance in potential study effect during the prediction of overall effect size (DerSimonian and Laird, 1986). The 'inverse variance' method was implemented to assign specific weighting to each study based on the variance of the effect, therefore more comprehensive studies with smaller standard error account for higher overall weights. This was done in attempt to lessen error or inaccuracy of the overall effect size in meta-analysis (Deeks et al., 2019). Individual standardised mean difference (SMD) was calculated for each cytokine from each study included in meta-analysis with 95% confidence intervals, in addition to an overall effect size for cytokine concentration favouring an increase in FEP or control (displayed by Z score) with overall 95% confidence intervals, and significance values noted ($p < .05$). SMD calculation was implemented due to the expectance of high study heterogeneity, and potential differences in sensitivity of cytokine measurements.

Enzyme Linked Immunosorbent Assays (ELISAs) are the gold standard technique for cytokine quantification, through the use of antibodies that have an affinity for target biological molecules and bind to quantify concentration of the analyte of interest. The sensitivity of the ELISA, which is the lowest possible detection concentration of the marker of interest, depends on the affinity of the anti-body, and is dependent on what manufacturer is selected and which ELISA kit is used. Therefore, the use of different kits between studies could cause discrepancies with regards to accurate cytokine quantification based on

differences in detection ranges, and the potential need to dilute samples to allow detection (Leng et al., 2008). Furthermore, ELISA-based methods may differ in the number of antibodies used to detect analytes, which effects the specificity of the assay dependent on the method used. Finally, the ability of the researcher to conduct an ELISA may affect the overall accuracy of cytokine quantification. Therefore, implementation of SMD will minimise the methodological differences between studies, and provide a standardised result on a uniform scale based on the variability within a given study, which may allow for a better comparison of results across studies (Higgins et al., 2019).

Inter study heterogeneity was evaluated by the Tau^2 and *Chi-square* tests. These tests determine if statistical heterogeneity is present between all studies for a given cytokine to determine if the change in cytokine concentration is similar across papers. The I^2 statistic was also calculated in order to display the percentage of variability in cytokine change across studies that is due to heterogeneity, which gives the reader an idea about the proportion of variance between studies. A low I^2 percentage would indicate that the change in cytokine in the FEP concentrations lie in close proximity with each other, suggesting a relatively consistent finding (Deeks et al., 2019). Data from RevMan 5.0 was displayed graphically via forest plots, with $p < .05$ denoting a significant effect of the diagnosis of FEP on cytokine concentration, when compared to an apparently healthy control population. The forest plot box relates to the relative effect of each study, the emanating lines represent confidence intervals, and the box size relates to the weight of the individual study. The diamond located at the bottom of the plot is an indicator of overall effect size, where if the diamond is not in contact with the line of no effect (vertical line at 0.0), a significant overall effect is present ($p < .05$).

In order to assess the potential relationship between cytokine concentration and FEP symptomology, mean, standard deviation, and sample size for cytokine concentration and

PANSS Negative from each study included in the meta-analysis, was entered into GraphPad Prism 8.0 for subsequent regression analysis. Cytokine concentrations (pg/ml) were transformed into values of fold change in comparison to control measurements which were normalised to 1.0, to allow for assessment of standardised values across studies. Data for each cytokine was also pooled to graphically display differences in cytokines that are present in FEP, in comparison to control.

5.0 Results

5.1 Paper Characteristics

Following PRISMA guidelines, a primary systematic search identified 1,360 studies. After the removal of 496 duplicate records, 864 studies were retrieved and the title and abstract screened against the identified inclusion criteria. Subsequently, 780 records were excluded. Full text analysis was then undertaken on the remaining 84 records. Articles were then excluded as follows: Condition not classified as FEP (n = 15), negative symptoms not used as a main outcome measure (n = 14), participants were not drug naïve (n = 12), study didn't measure/report peripheral cytokines (n = 9), no apparently healthy control group (n = 5), not in English language (n = 5), poster abstract only (n = 2), review article (n = 2). Finally, 6 studies were excluded due to duplication of the participant sample with another identified paper already included in this review (Borovcanin et al., 2013, Zhang et al., 2013, Borovcanin et al., 2015, Noto et al., 2015b, Borovcanin et al., 2018, Xiu et al., 2018). Where duplicate participant samples were identified, the paper that was included was selected based upon 1) largest panel of cytokines assessed, and 2) largest sample size, respectively. The study selection process is displayed in figure 7.

Data reviewed herein is included from FEP patients only. Where studies included data for participants in other disease groups (E.g. chronic high risk, chronic SCZ, depression) only data on participants with FEP and healthy controls was extracted. Altogether, 16 studies met the inclusion criteria for the current systematic review. Within the 16 included studies, the data from four studies was not provided in sufficient detail to be included in meta-analysis. Authors of these four studies were contacted and additional data was requested, but not received. These studies were therefore included in the narrative section of the review only. Reasons for exclusion from meta-analysis were as follows: standard deviation not given for cytokine values (n = 2), Over 50% cytokines at or below limit of detection (n = 1), actual cytokine values not given (n = 1). Furthermore, an additional two studies measured cytokines that were only featured once (IL-3, IL-18), therefore were not assessed via meta-analysis (n = 2).

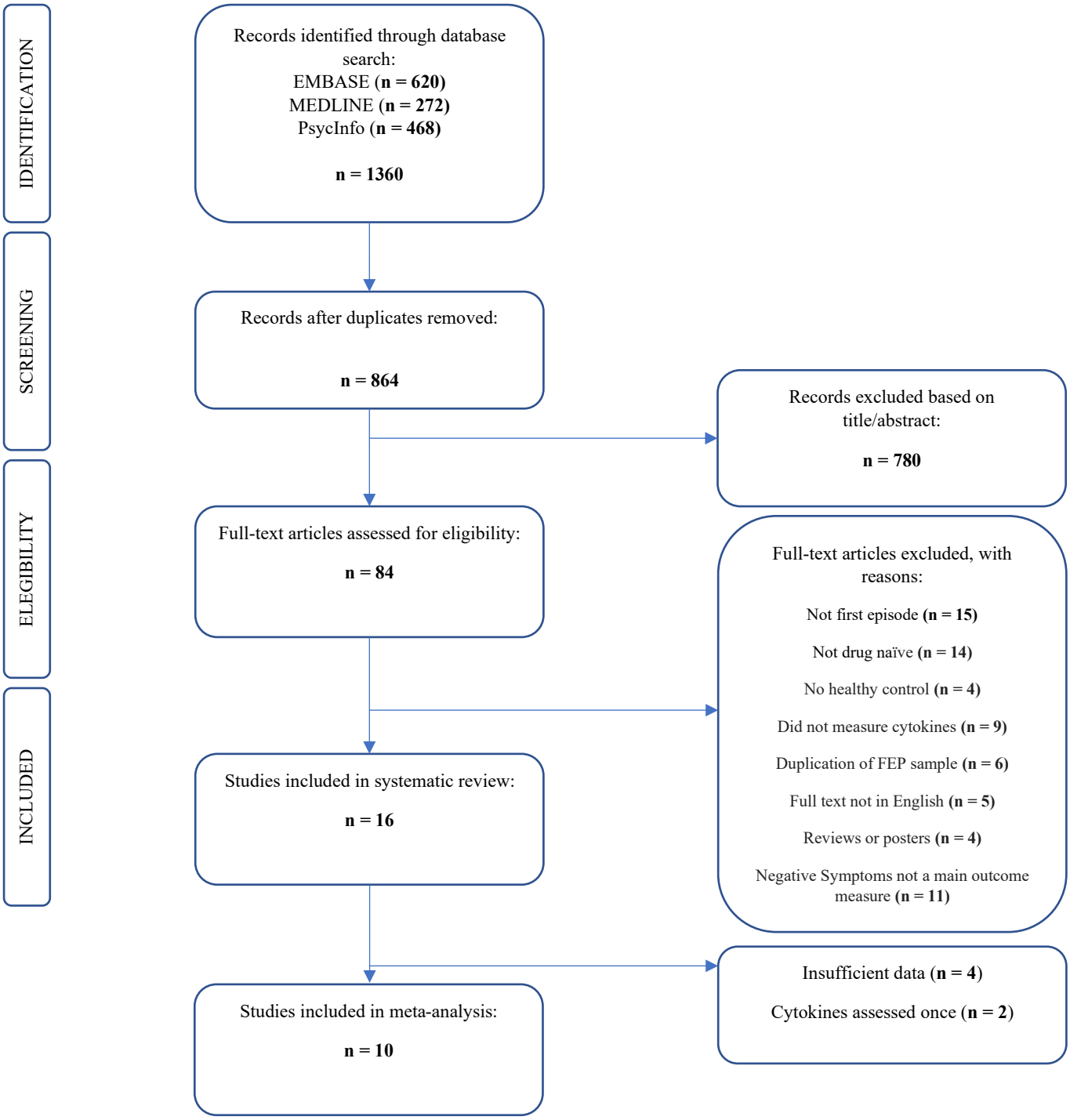


Figure 7. A flow chart displaying the selection process of studies included in the systematic review.

Table 2. Studies included in review

Author	Nationality	Drug Naïve FEP (n)	Healthy Control (n)	Cytokines Assessed	Methodology	Negative Symptoms Instrument	Comment
Borovcanin et al, 2012	Serbia	88	36	TNF-α, IFN-γ, IL-4, IL-6, IL-.10, IL-17, IL-27, TGF-β	ELISA	PANSS	Not included in meta-analysis
Crespo-Facorro et al, 2006	Spain	56	28	IL-12	ELISA	SANS	Included
Dai et al, 2020	China	83	60	IL-1β, IL-6	ELISA	PANSS	Included
Ding et al, 2014	China	69	60	IFN-γ, IL-6, IL-17	ELISA	PANSS	Included
Haring et al, 2015	Estonia	38	37	TNF-α, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10,	Sandwich Assay	PANSS	Included
Joaquim et al, 2018	Brazil	28	30	IL-1β	Multiplex Assay	PANSS	Included
Karanikas et al, 2017	Greece	25	23	TNF-α, TNF-β, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12	Immunoassay	PANSS	Not included in meta-analysis
Noto et al, 2019	Brazil	31	22	TNF-α, sTNF-R1/R2, IFN-γ, IL-1β, IL-1RA, IL-2, sIL-2r, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17,GM-CSF	Immunoassay	PANSS	Included

Table 2 continued. Studies included in review

Author	Nationality	Drug Naïve FEP (n)	Healthy Control (n)	Cytokines Assessed	Methodology	Negative Symptoms Instrument	Comment
Pesce et al, 2014	Italy	54	38	TNF-α, IL-1β, IL-2	ELISA	PANSS, SANS	Not included in meta-analysis
Petrikis et al, 2015	Greece	39	39	IL-2, IL-6, IL-10, IL-17, TGF-β	ELISA	PANSS	Not included in meta-analysis
Simsek et al, 2016	Turkey	30	26	TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-17	Flow Cytometry Bead Array	PANSS	Included
Xiu et al, 2012	China	78	78	IL-18	ELISA	PANSS	Included
Xiu et al, 2014	China	128	62	IL-10	ELISA	PANSS	Included
Yang et al, 2016	China	55	43	IL-3	ELISA	PANSS	Not included in meta-analysis
Zhu et al, 2018	China	69	61	TNF-α, IL-1β	ELISA	PANSS, SANS	Included
Zhu et al, 2020	China	119	135	TNF-α	ELISA	PANSS	Included

5.2 Meta-Analysis

Ten studies were included in the meta-analysis to assess cytokine concentrations in FEP, where cytokines were measured in two or more studies (Crespo-Facorro et al., 2008, Ding et al., 2014, Xiu et al., 2014, Haring et al., 2015, Şimşek et al., 2016, Joaquim et al., 2018, Zhu et al., 2018, Noto et al., 2019, Dai et al., 2020, Zhu et al., 2020). Data from the ten included studies provided information on 651 drug naïve FEP patients. The mean age of FEP patients was 25.18 ± 4.09 years, ranging from 14.7 to 29.07 years. The main diagnostic tools used to define FEP status were: DSM-IV (89.55%), ICD-10 (5.84%), DSM-V (4.61%). From the same ten studies, data was extracted for 521 apparently healthy control subjects, with a mean age of 26.01 ± 4.78 years, ranging from 14.5 to 32.9 years. Twenty-four different cytokines were assessed peripherally via ELISA, or similar assay. The following cytokines were assessed in more than one study: TNF-α, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-12, IL-17, (proinflammatory), IL-4, and IL-10 (anti-inflammatory). The data for each cytokine was included in a random-effects, pooled effect size model to determine which cytokines are significantly altered in FEP. After transformation into SMD to avoid potential differences in ELISA sensitivity, a random-effect, pooled-effect analysis suggested that four cytokines were significantly upregulated in the FEP population, when compared to control. A significant effect size was reported for IFN-γ (4 studies, 168 patients, SMD = 1.22, 95% CI 0.14 to 2.31, Z = .2.21), IL-6 (5 studies, 251 patients, SMD = 1.38, 95% CI 0.54 to 2.22, Z = 3.21), IL-12 (2 studies, 87 patients, SMD 3.43, 95% CI 2.12 to 4.73, Z = 5.15,), and IL-17 (3 studies, 130 patients, SMD = 0.72, 95% CI = 0.07 to 1.37, Z = 2.16) in patients with FEP, all retaining a significance p value of <.05. Six cytokines were not significantly altered in the FEP population when compared to control subjects. Non-significant effect sizes were reported for TNF-α (5 studies, 287 patients), IL-1β (4 studies, 218 patients), IL-2 (3 studies, 99 patients),

IL-4 (3 studies, 99 patients) IL-8 (2 studies, 69 patients), and IL-10 (4 studies, 227 patients). Forest plots are presented in Figure 8 broken down by study and cytokine of interest. In addition, Figure 10 presents data on cytokines that were measured in three or more studies, which included combined sample sizes over 100 patients. These findings suggest that IFN-γ, IL-6, and IL-17 are significantly elevated in the FEP population. The most replicated finding was for IL-6, which was increased within FEP in all five studies included in the meta-analysis.

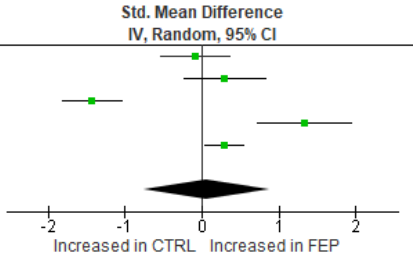
Heterogeneity measures were calculated in the current meta-analysis to assess the variation in outcomes between studies. The *Chi-square* test suggested that except for IL-2 and IL-4, there was significant heterogeneity in the effect size that is present between the studies for each cytokine assessed ($p < .05$). This means that there are differences in the magnitude of change of a given cytokine in the FEP population from study-to-study. This is explained to a greater extent by the I^2 statistic, which reported that with the exception of IL-2 and IL-4, there was high inter-study heterogeneity present for each cytokine included in the meta-analysis (equal to or above 80%, considerable heterogeneity) using the rule of thumb of 25%, 50%, and 75% as low, medium, and high heterogeneity, respectively. This statistic suggests that that for a given cytokine, the magnitude and direction of change that is present in FEP is not consistent from study-to-study, and the SMDs reported are widely dispersed around the overall mean of combined studies. This suggests that the studies as a group do not report a homogenous change in cytokine values in the FEP population, and a high proportion of fluctuation in cytokine concentration when comparing the studies is a result of inter-study heterogeneity or inconsistency. For example, in Figure 8 the I^2 statistic was calculated as 95% for TNF-α, which indicates that a high quantity of the differences in the reported magnitude of change in TNF-α concentrations per study in FEP are due to heterogeneity. Further examination of figure 8 support this finding, as the forest plot displays that some studies reported large

increases in TNF-α in FEP (Noto et al., 2019), whereas other studies saw smaller increases (Şimşek et al., 2016, Zhu et al., 2020), or even decreased TNF-α concentrations (Zhu et al., 2018). This can be applied to each cytokine included in the meta-analysis. Overall, the heterogeneity measures display that apart from IL-2 and IL-4, the change in peripheral cytokine concentration in FEP are not consistent across studies, and variations in findings can be expected due to high inter-study heterogeneity that goes beyond sampling error or chance, and may be a consequence of genuine differences between groups.

TNF-α

Study or Subgroup	FEP Mean	FEP SD	FEP Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Haring 2015	2.05	0.93	38	2.16	1.41	37	20.0%	-0.09 [-0.54, 0.36]	2015
Simsek 2016	9.22	22.2	30	4.2	4.9	26	19.6%	0.30 [-0.23, 0.83]	2016
Zhu 2018	8.2	2	69	15.4	7	61	20.4%	-1.43 [-1.82, -1.04]	2018
Noto 2019	4.12	0.22	31	3.8	0.26	22	19.1%	1.33 [0.72, 1.93]	2019
Zhu 2020	2.21	0.33	119	2.11	0.36	135	20.9%	0.29 [0.04, 0.54]	2020
Total (95% CI)			**287**			**281**	**100.0%**	**0.06 [-0.75, 0.87]**	

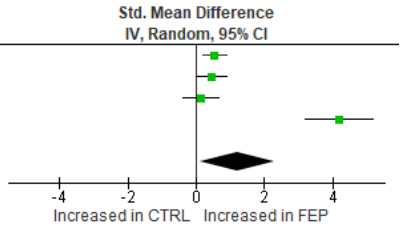
Heterogeneity: Tau² = 0.80; Chi² = 77.34, df = 4 (P < 0.00001); I² = 95%
Test for overall effect: Z = 0.15 (P = 0.88)



IFN-γ

Study or Subgroup	FEP Mean	FEP SD	FEP Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Ding 2014	509.73	144.44	69	438.54	113.7	60	26.4%	0.54 [0.19, 0.89]	2014
Haring 2015	0.35	0.13	38	0.3	0.09	37	25.9%	0.44 [-0.02, 0.90]	2015
Simsek 2016	2.9	15.6	30	1.03	7.1	26	25.5%	0.15 [-0.38, 0.67]	2016
Noto 2019	7.03	0.45	31	4.97	0.53	22	22.1%	4.19 [3.20, 5.18]	2019
Total (95% CI)			**168**			**145**	**100.0%**	**1.22 [0.14, 2.31]**	

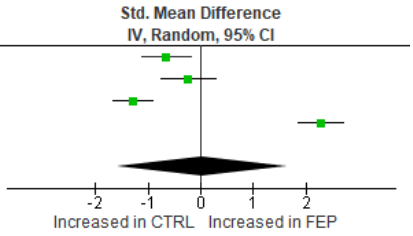
Heterogeneity: Tau² = 1.13; Chi² = 53.47, df = 3 (P < 0.00001); I² = 94%
Test for overall effect: Z = 2.21 (P = 0.03)



IL-1β

Study or Subgroup	FEP Mean	FEP SD	FEP Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Haring 2015	1.35	0.7	38	1.88	0.89	37	25.0%	-0.66 [-1.12, -0.19]	2015
Joaoquim 2018	0.28	0.28	28	0.39	0.58	30	24.8%	-0.24 [-0.75, 0.28]	2018
Zhu 2018	1.7	0.2	69	8.3	7.5	61	25.1%	-1.28 [-1.66, -0.90]	2018
Dai 2020	69.48	24.75	83	24.77	8.09	60	25.0%	2.27 [1.84, 2.70]	2020
Total (95% CI)			**218**			**188**	**100.0%**	**0.03 [-1.60, 1.65]**	

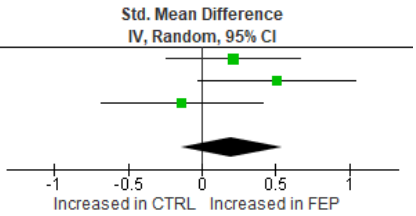
Heterogeneity: Tau² = 2.68; Chi² = 161.20, df = 3 (P < 0.00001); I² = 98%
Test for overall effect: Z = 0.03 (P = 0.98)



IL-2

Study or Subgroup	FEP Mean	FEP SD	FEP Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Haring 2015	3	0.65	38	2.85	0.73	37	39.1%	0.21 [-0.24, 0.67]	2015
Simsek 2016	20	13	30	13.7	11.3	26	31.0%	0.51 [-0.03, 1.04]	2016
Noto 2019	2.34	0.61	31	2.43	0.71	22	29.9%	-0.14 [-0.68, 0.41]	2019
Total (95% CI)			**99**			**85**	**100.0%**	**0.20 [-0.14, 0.54]**	

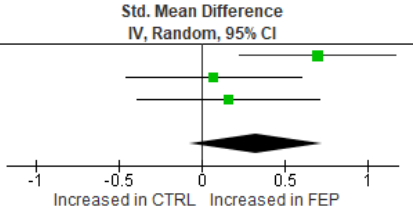
Heterogeneity: Tau² = 0.02; Chi² = 2.72, df = 2 (P = 0.26); I² = 27%
Test for overall effect: Z = 1.15 (P = 0.25)



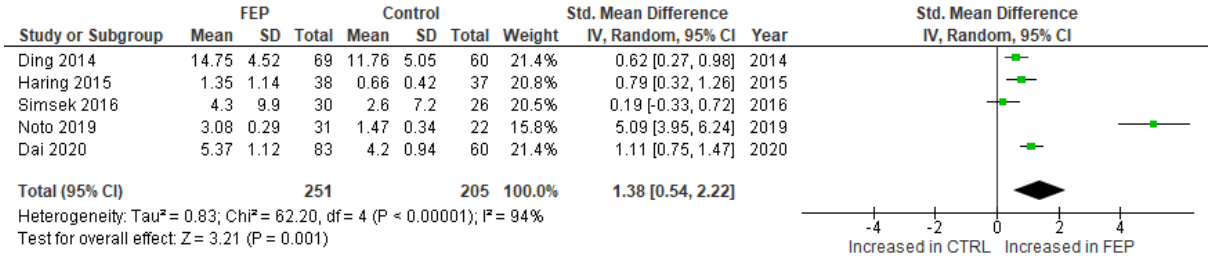
IL-4

Study or Subgroup	FEP Mean	FEP SD	FEP Total	Control Mean	Control SD	Control Total	Weight	Std. Mean Difference IV, Random, 95% CI	Year
Haring 2015	1.73	0.55	38	1.4	0.37	37	36.7%	0.69 [0.23, 1.16]	2015
Simsek 2016	30.5	5.3	30	30.1	5.7	26	32.4%	0.07 [-0.45, 0.60]	2016
Noto 2019	13.75	2.24	31	13.36	2.64	22	30.9%	0.16 [-0.39, 0.71]	2019
Total (95% CI)			**99**			**85**	**100.0%**	**0.33 [-0.07, 0.73]**	

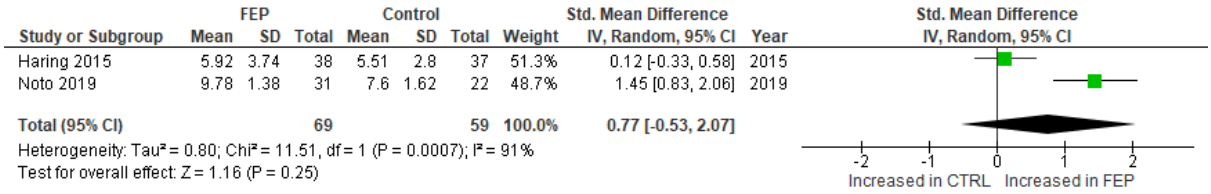
Heterogeneity: Tau² = 0.06; Chi² = 3.64, df = 2 (P = 0.16); I² = 45%
Test for overall effect: Z = 1.61 (P = 0.11)



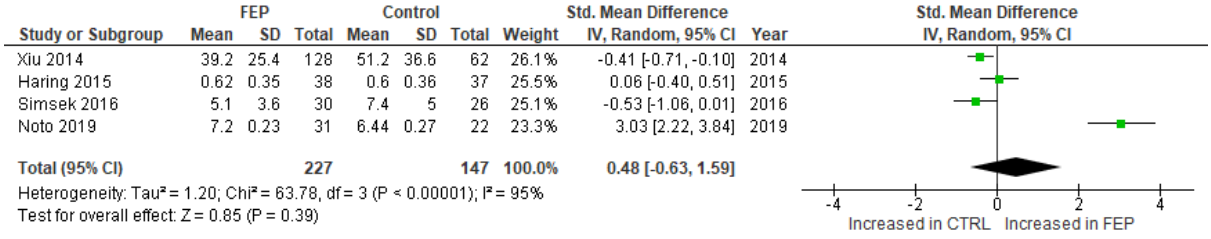
Il-6



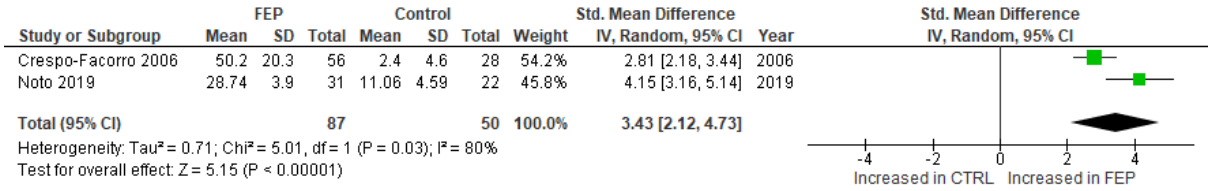
Il-8



IL-10



IL-12



IL-17

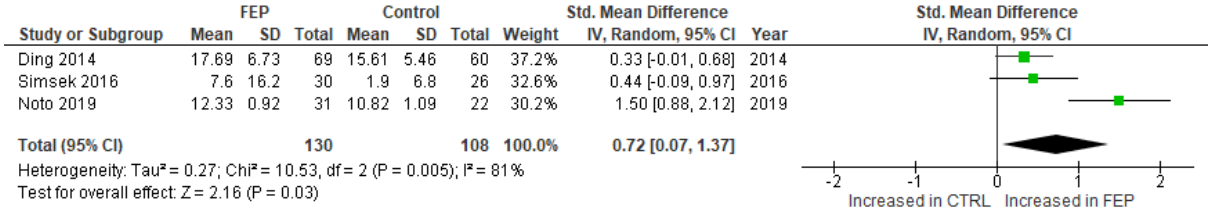


Figure 8. Forest plots displaying the standardised mean difference between FEP and control for each cytokine. Tests for heterogeneity in effect size for each cytokine, as well as overall effect size given for each cytokine. Also plotted graphically per cytokine, with the box size relating to the weight of each study, position relating to the difference in FEP vs control, and the emanating lines relating to the 95% confidence intervals. The diamond represents overall effect size.

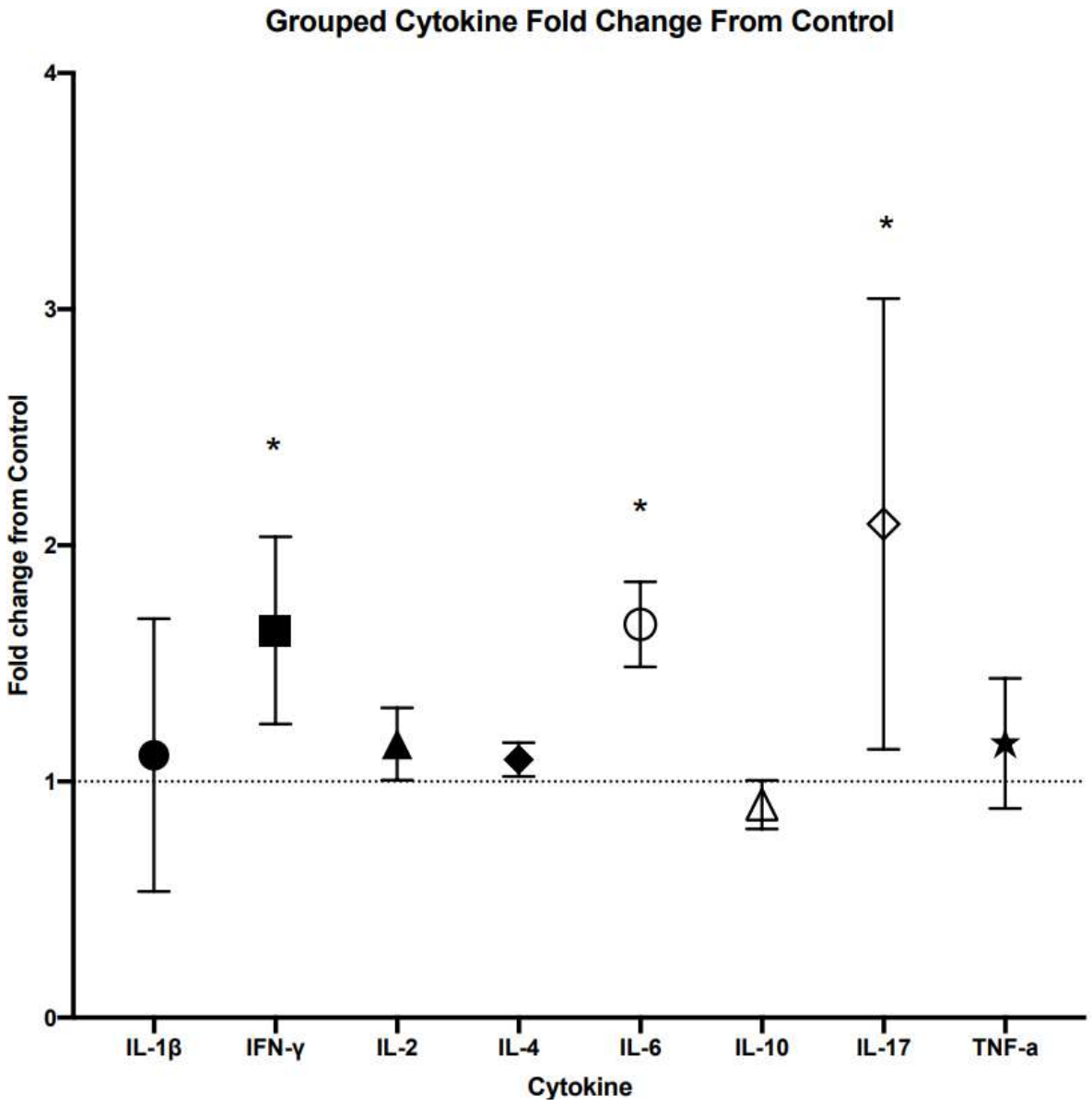


Figure 9. A Scatter plot displaying the fold-change for each cytokine measured in 3 or more studies with over 100 participants, with reference to control subjects normalised to 1.0. The horizontal line represents control, symbols represent each cytokine fold change in FEP with SD, '' denotes a significant difference.*

5.3 Cytokine & Negative Symptom Analysis

Significant correlations between the PANSS Negative sub-score and change in inflammatory markers were reported seven times, in six different studies, with reference to 6 different cytokines. An overview of these findings is present in Table 3. The main analytical methods utilised for the assessment of this relationship were the Pearson's correlation, and multiple

regression. According to the correlation strength estimates proposed by Cohen (1998), a strong positive correlation between IL-4 and the PANSS Negative sub-score were reported (r = .67). Moderate correlation strengths were reported for IL-6 (r = .48), IL-2 (r = .409), TNF-α (r = .37), and the PANSS Negative sub-score. Two correlations were reported between IL-10 and the PANSS Negative subscore, including a strong negative correlation (r = -.65) reported by Simsek et al (2016), and a weak-to-moderate negative correlation (r = -.195) reported by Xiu et al, (2014). Dai et al, (2020) reported a significant and positive β coefficient for the relationship between IL-1β and the PANSS Negative sub-score (β = .486**)**, which suggests that an increase in IL-1β caused an increase in the PANSS Negative scale. No other significant relationships were reported ($p < .05$). IL-10 was reported as correlated with the PANSS Negative subscale in 50% of studies measuring this cytokine. Due to the absence of sufficient supplementary data and lack of correspondence by the majority of authors, no larger scale analysis could take place.

Cytokine	Author	Cytokine (Pg/mL)	PANSS Negative	Analytical Method	Findings	Comments
IL-1β	Haring et al, 2015	1.35	22.97	Pearson Correlation	r = .232	NS
	Joaquim et al, 2018	0.28	18.00	Pearson Correlation	Not stated	NS
	Zhu et al, 2018	1.70	25.60	Partial Correlation	Not stated	NS
	Dai et al, 2020	**69.48**	**22.16**	Multivariate Regression	**β = .486**	***Positive**
IFN-γ	Ding et al, 2014	509.73	18.23	Pearson Correlation	r = .074	NS
	Haring et al, 2015	0.35	22.97	Pearson Correlation	r = .162	NS
	Simsek et al, 2016	2.90	26.30	Pearson Correlation	r = .30	NS
	Noto et al, 2019	7.03	20.59	Multiple Regression	Not stated	NS
IL-2	**Haring et al, 2015**	**3.00**	**22.97**	Pearson Correlation	**r = .409**	***Positive**
	Simsek et al, 2016	20.00	26.30	Pearson Correlation	r = -.12	NS
	Noto et al, 2019	2.34	20.59	Multiple Regression	Not stated	NS
IL-4	Haring et al, 2015	1.73	22.97	Pearson Correlation	r = .073	NS
	Simsek et al, 2016	**30.5**	**26.30**	Pearson Correlation	**r = .67**	***Positive**
	Noto et al, 2019	13.75	20.59	Multiple Regression	Not stated	NS
IL-6	Ding et al, 2014	14.75	18.23	Pearson Correlation	r = .168	NS
	Haring et al, 2015	1.35	22.97	Pearson Correlation	r = .227	NS
	Simsek et al, 2016	4.30	26.30	Pearson Correlation	r = -.57	NS
	Noto et al, 2019	**3.08**	**20.59**	Multiple Regression	**r = .48**	***Positive**
	Dai et al, 2020	5.37	22.16	Multivariate Regression	β = .130	NS
IL-8	Haring et al, 2015	5.92	22.97	Pearson Correlation	r = .04	NS
	Noto et al, 2019	9.78	20.59	Multiple Regression	Not stated	NS
IL-10	**Xiu et al, 2014**	**39.20**	**18.90**	Pearson Correlation	**r = -.195**	***Negative**
	Haring et al, 2015	0.62	22.97	Pearson Correlation	r = .097	NS
	Simsek et al, 2016	**5.10**	**26.30**	Pearson Correlation	**r = -.65**	***Negative**
	Noto et al, 2019	7.20	20.59	Multiple Regression	Not stated	NS
IL-12	Crespo-Facorro et al, 2006	50.2	10.11	Pearson Correlation	Not stated	NS
	Noto et al, 2019	28.74	20.59	Multiple Regression	Not stated	NS
IL-17	Ding et al, 2014	17.69	18.23	Pearson Correlation	r = .204	NS
	Simsek et al, 2016	7.60	26.30	Pearson Correlation	r = .23	NS
	Noto et al, 2019	12.33	20.59	Multiple Regression	Not stated	NS
TNF-a	Haring et al, 2015	2.05	22.97	Pearson Correlation	r = .034	NS
	Simsek et al, 2016	9.20	26.30	Pearson Correlation	r = -.46	NS
	Zhu et al, 2018	8.20	25.60	Partial Correlation	Not stated	NS
	Noto et al, 2019	4.12	20.59	Multiple Regression	Not stated	NS
	Zhu et al, 2020	**2.21**	**26.89**	Multiple Regression	**r = .37**	***Positive**

Table 3. A table reporting the findings of each individual study, per inflammatory marker, with regards to a correlation between cytokine and PANSS Negative Sub-score. Correlations reported as 'r' value, or β coefficient dependant on how each study reported results. Significant correlations marked in bold with an asterisk '', followed by the direction of the relationship between the two variables. NS, not significant.*

5.4 Additional Data Ruled Out of Meta-Analysis

IL-3 was assessed in FEP patients in only one study within this review, conducted by Yang et al., (2016). This study reported significantly decreased IL-3 concentrations in FEP patients, when compared to healthy controls ($p < .01$), however the change in IL-3 in FEP patients was not associated with changes in the PANSS negative score or any other symptomology. Xiu et al., (2012) assessed IL-18 in FEP, and similarly, this was the only study included in the present review to assess this cytokine. No significant difference in IL-18 concentration was found when compared to healthy controls, and no correlation was reported with the PANSS Negative score or any other symptomology ($p > .05$).

The study by Borovcanin et al., (2012) was excluded from the meta-analysis as the data was presented as standard error of the mean (SEM) rather than standard deviation. The use of SEM would likely skew any meta-analysis, and following calculation of SD from the data provided, this was indeed the case. Borovcanin et al, (2012) reported significantly elevated TGF-β in FEP when compared to healthy controls, with no significant difference reported for IFN-γ, IL-4, IL-6, IL-17, IL-27, and concentrations below detection limit for IL-10 and TNF-α. Borovcanin et al., (2012) also reported that there was no relationship found between cytokines and symptomology. The study by Pesce et al., (2014) was excluded from the meta-analysis as actual values for cytokines were not stated, and no correspondence was made after an attempt to contact the authors. However, this study suggested significantly that IL-1β, IL-2, and TNF-α were increased in FEP patients when compared to control, and a significant

positive relationship between IL-1β and SANS scores was presented, in which increasing IL-1β was associated with increased SANS scores, implying more severe negative symptomology.

The study by Petrikis et al., (2015) was excluded from the meta-analysis due to most data points presented being at or below the limit of detection of the ELISA. However, the study presented data to suggest a significant increase in IL-2 and IL-6 in FEP, though no relationship with negative symptomology was evident. Finally, the study by Karanikas et al., (2017) was excluded from the meta-analysis as sufficient cytokine data was not available. This group reported significantly increased TNF-α, TNF-β, IFN-γ, and IL-4 in FEP, but no correlation with any symptomology was presented.

6.0 Discussion

General Findings

The present systematic review is the first to bring together and analyse data on the relationship between proinflammatory cytokine concentrations in FEP, and negative symptomology as assessed by the PANSS or SANS questionnaires. The results presented from the meta-analysis conducted within the review suggest that IFN-γ, IL-6, IL-12, and IL-17 are all significantly increased in the FEP population. This is indicative of a perturbed inflammatory response in the very early stages of psychosis. The significant increases in IFN-γ, IL-6 and IL-12 concentration had an associated large effect size (range: $d = 1.2 – 3.43$) with reference to Cohen's effects estimates (Cohen, 1988). IL-17 concentration was also increased but had an associated moderate effect size ($d = 0.72$). Although sufficient raw data for the PANSS and SANS symptom scales was absent from many studies, and thus

regression analysis was not possible, significant positive relationships between PANSS Negative and IL-1β, IL-2, IL-4, IL-6, and TNF-α were identified from cohort data provided in each study independently. Of the cytokines reviewed from a narrative perspective, IL-4 and PANSS Negative ($r = .67$, $p < .05$) appear most closely related. Furthermore, a significant negative relationship between IL-10 and the PANSS Negative subscale was reported in two studies. Overall, the lack of complete data sets supplied meant that a full meta-analysis on the relationship between cytokines and symptomology could not be conducted. More research studies with full publication of supplementary data are required to allow a more accurate and thorough analysis of the relationships between cytokine concentration and negative symptoms.

A significant increase in proinflammatory cytokines within a FEP population as identified herein, is in agreement with previous meta-analyses conducted in FEP and SCZ investigating disease related changes in cytokine concentration (Miller et al., 2011, Upthegrove et al., 2014, Frydecka et al., 2018, Fraguas et al., 2019, Pillinger et al., 2019). The outcome of this review further supports the hypothesis of a dysregulated immune system as a key component of SCZ pathology. Specifically, our meta-analysis was in line with various findings by Miller et al., (2011) and Upthegrove et al., (2014), to identify specific increases in IFN-γ, IL-6, and IL-12, within the FEP population. Perturbed concentrations of these cytokines that have been suggested as important in SCZ pathology, may be a consequence of mechanistic action of environmental stressors that are known to contribute to a vicious cycle of positive feedback in immune cells throughout a lifetime. This can manifest in chronic neuroinflammation, as described in the 'vulnerability-stress-inflammation' model (Müller, 2018). For example, dysregulated microglial production of IL-6 that is a hallmark of SCZ, may facilitate an imabalance of the Th1/Th2 immune response. Ultimately, this can lead to the subsequent upregulation in IFN-γ, IL-2, and IL-12, alongside the chronic activation of microglia and

astrocytes that underpin these persistent increases in central inflammation (Smith and Maes, 1995, Monji et al., 2009, Miller et al., 2011). This suggests that cytokines do not work independently and may operate through an inflammatory network to reciprocally upregulate other cytokines and exacerbate inflammation, and may explain why cytokines appear to be upregulated simultaneously in patterns and not in isolation (Goldsmith et al., 2016), both in the current meta-analysis and elsewhere. It is generally accepted that the changes in cytokines in the peripheral system are representative of those occurring in the CNS (Wang and Miller, 2018). Therefore, the current meta-analysis is in line with reviews that propose chronic central inflammation and microglial activation in FEP and SCZ.

Although the current meta-analysis was largely in agreement with previously conducted systematic reviews in similar patient groups, there were some key differences in the concentrations that have been previously reported, for example non-altered IL-1β and TNF-α concentrations, which could be due to multiple factors. Firstly, we adopted a flexible yet specific definition of FEP that was most suitable to accurately represent early stages of disease by inclusion criteria based on data that is available in published studies, which consisted of an illness duration of up to five years. This offers a more accurate representation of the early psychosis spectrum that includes individuals that are in the infancy of disease, as well as patients that have more advanced pathology but still classify as FEP. This is key, as the 'critical period' of deterioration in FEP is proposed to occur for up to five years, therefore it is logical to assess cytokines over this entire timeframe (Birchwood et al., 1998). This could be important as some cytokines are identified as a 'state' cytokines which are increased upon acute exacerbations of disease (Miller et al., 2011), therefore if a longer time period has elapsed since the first psychotic episode before peripheral measurements are taken, these cytokines may have somewhat normalised in concentration. This may explain why we failed to observe a consistent increase in IL-1β in the current meta-analysis. The flexible definition

that was selected for the current review was most suitable to attempt to accurately classify early psychosis. This is because, data on specific time elapsed since first episode may be difficult to obtain if not disclosed in the published papers, and often different criteria are used to define FEP across studies. Whereas, data on duration of illness, absence of previous clinical presentation, and drug naivety are often readily available in published studies and represent accurate measurements of early disease progression. This may differ from criteria used in other systematic reviews and may explain some of the differences in findings that are present in the current review.

Additionally, within the current review smoking was not defined as part of the exclusion criteria. This is because sufficient data on smoking status, as well as smoking quantity/habits are often not disclosed or measured by all authors, which was the case in the current systematic review, and lack of correspondence meant that this data could not be obtained upon request. Further, even if smoking values are stated, it may be questioned whether self-report questionnaires can be regarded as valid measurements if individuals are reporting on illegal substances, for example cannabis which is illegal in most countries. Tobacco and cannabis use is increasingly common within the FEP population across the lifespan, with tobacco smoking prevalence estimated at 62% (De Leon and Diaz, 2005), and lifetime use of cannabis estimated at 42.1% (Green et al., 2005). This could be important, as smoking may have immunosuppressive effects with regards to inflammation and cytokine concentration (Zhang et al., 2008), though more research is needed to clarify this effect. However, SCZ is a heterogeneous disease by nature, and pathological changes that occur are thought to be a consequence of individual genetic and environmental differences that converge. Therefore, differences in cytokines expression may be identified between individuals as well as on a larger scale between populations, but still within an overall common proinflammatory status that is characteristic of psychosis. The impact of genetic and environmental factors

underlying psychosis may well reflect the differences that have been identified within the current meta-analysis when compared to other reviews in similar patient groups.

Pro-Inflammatory Cytokines and Negative Symptoms

Data presented herein suggest a significant association of multiple cytokines with the PANSS Negative and SANS symptomology sub-scales. In terms of proinflammatory cytokines, IL-6 may be considered one of the most well characterised and well-studied cytokines in SCZ pathology and has been associated with negative symptoms in multiple different stages of disease. However, due to the pleiotropic nature of IL-6, studies that report increases in this cytokine must be interpreted with caution, as this could equally represent an upregulation of anti-inflammatory action in acute phases of disease in order to counteract the large increases in inflammation that are observed. Therefore, the range of correlations, and in some cases the lack of any apparent relationship between IL-6 concentration and symptomology may be evidence of the pleiotropic effects of IL-6, where this cytokine may exert pro-and anti-inflammatory effects both peripherally and centrally in attempts to regulate inflammation.

IL-6 plays a fundamental role in the proliferation of other immune cells, hormone-like effects during exercise, induction of acute phase proteins, and most importantly exerts potent effects within the innate immune system (Pedersen et al., 2001, Kishimoto, 2010, Hunter and Jones, 2015). In response to cell damage or identification of pathogens, IL-6 is rapidly upregulated by immune cells and functions to notify the body of the stressor, and to initiate the immune response for removal of pathogens via phagocytosis and regulated ROS production (Choy and Rose-John, 2017). However, dysregulation of IL-6 production can prolong the proinflammatory effects that are usually considered protective, and contribute to neuroinflammation (Hunter and Jones, 2015). Indeed, IL-6 has been reported as upregulated in FEP and acute relapse SCZ, and this was also reported in the current meta-analysis, therefore reaffirming its' role as a 'state' cytokine that increases during acute psychotic

episodes of disease (Miller et al., 2011). Overall, 4 out of 5 studies that investigated IL-6 in the current meta-analysis, noted a positive correlation between IL-6 concentration and the magnitude of negative symptoms, although only the data reported by Noto et al. (2019), reached statistical significance, as shown in Table 2. Noto et al. (2019), suggested a significant and positive relationship between IL-6 and the PANSS Negative sub-score, and reported that IL-6 alone accounted for 23% variance in the negative symptomology score. Interestingly, this group categorised cytokines based on their function profile, and found intercorrelations between various cytokines that were upregulated together in FEP, which may further suggest that cytokines function in patterns to exacerbate overall inflammation. Interestingly, Simsek et al. (2016), reported a moderate negative correlation between IL-6 and negative symptoms that was on the borderline of significance within an early-onset adolescent SCZ cohort. This may be evidence of the pleiotropic anti-inflammatory effects of IL-6, and lends support to the hypothesis that IL-6 dysregulation in early life may be important in accelerating deterioration into disease, and aberrations in IL-6 concentration may well cause the presentation of negative symptoms earlier in disease pathology.

These findings are in keeping with multiple studies conducted elsewhere that have investigated IL-6 concentrations in various stages of SCZ pathology. For example, Stojanovic et al. (2014), investigated IL-6 concentrations in an acute psychotic disorder group comprised of SCZ patients, and an ‘at risk of psychosis’ population, which represent two different stages of SCZ pathology. This group reported a significant upregulation in IL-6 in both groups compared to control subjects, even after controlling for potential cofactors of body mass index, habitual smoking, and alcohol intake. Furthermore, the IL-6 concentration in both groups within this study were significantly and positively associated with the negative symptoms when assessed by the PANSS questionnaire. This may suggest a prominent role for IL-6 in both acute and chronic stages with regards to negative symptomology, in which

increasing IL-6 may correspond to increased severity of negative symptoms through a predominantly inflammatory mechanism (Stojanovic et al., 2014).

IL-6 is suggested to facilitate transition from an acute inflammatory response, to more chronic neuroinflammation through the recruitment of other proinflammatory cytokines (TNF-α, IL-1β etc.) and acute phase proteins that may remain elevated even after an acute psychotic episode (Miller et al., 2011). Therefore, IL-6 is proposed as a potential central mediator of overall disease risk through the generation of a chronic proinflammatory microenvironment that may increase the risk of later psychotic episodes (Khandaker et al., 2014). This may facilitate the development of negative symptoms as IL-6 upregulation can compromise and alter the blood-brain barrier, which may promote migration of inflammatory markers into the CNS to promote neuroinflammation (Stojanovic et al., 2014). Overall, this could lead to central oxidative stress, increased production of other cytokines, and subsequent alterations in brain volume, all of which been associated with negative symptoms in SCZ (Kirkpatrick and Miller, 2013). Specifically, reductions in hippocampal volume which have been reported as related to the emergence of negative symptoms in FEP, have been directly predicted by increases in IL-6 concentrations in FEP (Mondelli et al., 2011). Once the blood-brain barrier is infiltrated, IL-6 may also be involved in the cytokine induced degradation of tryptophan to kynurenic acid, therefore may indirectly contribute to negative symptom severity through disturbance of glutamatergic, dopaminergic, and serotonergic transmission (Kindler et al., 2019). Interestingly, elevated IL-6 has been reported in individuals with deficit SCZ which is characterised by enduring negative symptoms, when compared to a non-deficit SCZ group, therefore ascribing importance to this cytokine for long term negative symptomology in which inflammation may play a fundamental role. Prolonged neuroinflammation as a consequence of IL-6 action may lead to the downregulation in activation of striatal regions that have been suggested to underpin motivational anhedonia in

particular (Goldsmith et al., 2018, Goldsmith and Rapaport, 2020). This is supported by a contemporary study included in the current systematic review, as Dai et al. (2020), reported significantly increased IL-6 levels within a SCZ subgroup which were predominantly characterised by negative symptoms, when compared to both a group characterised by positive symptoms, and a control group. This may further implicate inflammation as a specific characteristic of deficit symptoms and negative symptomology specifically, in SCZ.

Mechanistically, increases in IL-6 are typically accompanied by IL-1β, which is known to induce IL-6 release. IL-1β is secreted by astrocytes and microglia centrally and has been reported as increased following infection as a key component of host-defence. IL-1β functions to activate immune cells and promote the production of other proinflammatory cytokines, and is implicated heavily in SCZ pathology (Goldsmith et al., 2016). Within the current systematic review, two studies identified significant relationships between IL-1β and negative symptomology (Pesce et al., 2014, Dai et al., 2020). Dai et al. (2020), reported significant increases in IL-1β concentrations that were positively associated with the PANSS Negative subscale in a general FEP population, as well in as a specific subgroup of FEP patients characterised by negative symptoms specifically. This was reported alongside an increase in IL-6 and may lend support to the notion of a cytokine-mediated emergence of negative symptoms. These findings are in line with a previous study by Pesce et al. (2014), which was also included in the current review, which reported a significant relationship between peripheral IL-1β concentrations and the SANS negative symptom questionnaire. It is suggested that IL-1β may be passively transported to the brain, or alternatively released directly within the brain by microglia and astrocytes (Monji et al., 2009). Therefore IL-1β may be in part responsible for the exacerbation of central inflammation and persistent neurodegenerative processes (Rothwell and Luheshi, 2000, Pesce et al., 2014). Rodent based studies have suggested IL-1β as an important factor for the activation the hypothalamic-

pituitary-adrenal axis and subsequent presentation of anhedonia, which may emphasize the importance of perturbations in IL-1β concentration for both SCZ and depression (Lawson et al., 2013).

IL-1β may be involved in the dysregulation of inflammation that is present in SCZ due to its' rapid response to stress and ability to induce IL-6 and further Nf-κB activation. Specifically, the combined effects of IL-1β, IL-6, and TNF-α have been suggested as crucial in activation of IDO, as well as TDO and enzymes of the kynurenic acid pathway (Campbell et al., 2014). Interestingly, one study included in the current review reported a significant positive correlation of IL-1β concentration with peripheral kynurenine, though no direct correlation between IL-1β and the PANSS Negative sub-score was reported. There was however a significant correlation between kynurenine concentration and negative symptoms in this study, which may suggest an indirect effect of IL-1β within early FEP that facilitates the alternate metabolism of serotonin towards kynurenine, and may reflect increased KYNA production in the CNS (Joaquim et al., 2018). This may in turn lead negative symptom presentation through hypofunction of glutamate. However, some reports suggest that IL-1β shows more of an association with negative symptoms in chronic type SCZ, as opposed to recently established FEP. For example, within the current review Zhu et al. (2018), reported a positive correlation between IL-1β and the PANSS Negative sub-score in chronic SCZ patients but failed to replicate this finding in a first episode population. Additionally, Haring et al. (2015), found no significant correlation of IL-1β to negative symptomology in FEP patients prior to any antipsychotic treatment. This may implicate the duration of illness as a potential mediator of the effects of IL-1β on negative symptoms, as this cytokine may downregulate after exacerbations of disease.

Alongside IL-6 and IL-1β, TNF-α is also one of the most consistently upregulated 'trait' cytokines in FEP and SCZ, and represents a 'trait' cytokine (Miller et al., 2011) that has been

associated with negative symptomology. TNF-α is a proinflammatory cytokine that is upregulated rapidly by glia and astrocytes in response to pathogens and stressors, and is involved in apoptosis and inflammation aggravation (Kalliolias and Ivashkiv, 2016). Given its' 'trait' status, prolonged elevations in TNF-α concentration that have been found in SCZ have been suggested to contribute to negative symptom severity and may also be important with regards to longer term deficit symptomology. Within the current systematic review, Zhu et al. (2020), reported a significantly elevated TNF-α concentration and described a positive relationship between TNF-α levels and negative symptom score in first episode patients, which remained significant after Bonferroni correction. One mechanism suggested to explain this relationship involved the potential for TNF-α induced downregulation in the activation of striatal regions involved in reward anticipation, which may give rise to anhedonic behaviour (Zhu et al., 2020). Evidence presented elsewhere involving earlier and late stage SCZ pathology has reaffirmed the link between TNF-α and negative symptomology, involving dysregulation of the neurotransmitter pathways and cytokine-mediated reductions in activation of key brain areas (Goldsmith and Rapaport, 2020).

Interestingly, research conducted elsewhere suggested that TNF-α was able to predict the slope of negative symptoms over time in a chronic high risk population, where higher TNF-α concentration were associated with larger increases in negative symptoms after a 1-year follow up period, independent of depression (Goldsmith et al., 2019). This provides evidence to suggest that TNF-α concentrations may be pertinent to increased severity of negative symptomology in early disease stages and may be relevant for the progression in severity of these symptoms as disease develops. Earlier studies conducted by Goldsmith et al. (2018), reported that TNF-α was significantly upregulated in a deficit SCZ subgroup, when compared to a non-deficit subgroup and control subjects, which may further implicate TNF-α and inflammation as a specific characteristic of enduring negative symptomology in particular.

Specifically, TNF-α was significantly associated with alogia and blunted affect after controlling for medication effects in a chronic deficit SCZ population (Goldsmith et al., 2018). Other contemporary research has also reported significantly increased TNF-α in deficit SCZ populations when compared to non-deficit and control groups, and significant increases in TNF-α that could able to predict the severity of negative symptoms (Maes et al., 2020).

The most intriguing finding is that although Zhu et al. (2020), reported a significant relationship between TNF-α and negative symptoms, a study conducted by the same group two years earlier found no such correlation. In fact, this study reported FEP patients as having a significantly lower TNF-α concentration than chronic SCZ and healthy control patients (Zhu et al., 2018). Overall, four out of five studies included in the systematic review did not report a significant relationship between TNF-α and negative symptoms. It could be suggested that TNF-α may exert more long-term effects that manifest in deficit-type SCZ, and these changes may be visible as early as at-risk and FEP. However, the current inconsistencies in studies in reporting the prospective relationship between TNF-α and negative symptoms means that no definitive conclusions can be made, and more longitudinal research need be completed to reveal any potential association.

The final proinflammatory cytokine that was reported to display an association with negative symptoms in the current review was IL-2. This cytokine is involved in promotion of the proinflammatory response through upregulation of T-cells that can induce further inflammation. IL-2 may also be implicated in the inhibition of Th17 cytokines, and has been linked to neuroregulatory functions in the brain (Abbas et al., 2018). In the current review, IL-2 displayed a trend of an increase in FEP across studies, although was found to be not significant. In terms of the relationship between IL-2 and negative symptoms in FEP, one of three studies included in meta-analysis reported a significant and positive relationship.

Although not described in the published version, Haring et al. (2015), provided sufficient supplementary data in order for us to conduct our own statistical analysis, in which we found a significant correlation between IL-2 and the PANSS Negative sub-score in a FEP cohort before initiation of any antipsychotic treatment ($r = .409$ $p = .01$), although the absolute concentration of IL-2 was not significantly different from control subjects. It has been suggested that IL-2 may be capable of modulating dopaminergic and serotonergic pathways in key brain areas (Pesce et al., 2014), where dysregulation of neurotransmission may be important for the genesis of negative symptoms in SCZ. Further, the ability of IL-2 to induce further cytokine production may represent an indirect pathway that contributes to negative symptomology through associated neuroinflammatory effects. However, a study conducted in a chronic medicated SCZ cohort suggested that IL-2 levels were significantly lower in SCZ, and negatively correlated with PANSS Negative constructs including 'blunted affect', 'difficulty in abstract thinking', and 'poor rapport' (all <.05) (Asevedo et al., 2014). This finding contradicts the reports in FEP and suggests that lower IL-2 contributes to increased negative symptom severity. However, it must be acknowledged that these patients were under chronic medication with atypical antipsychotics including olanzapine and clozapine, which have been previously suggested to modulate microglial activation and cytokine concentrations, including IL-2 (Hu et al., 2012, Stapel et al., 2018).

Studies conducted elsewhere have reported increased concentration of the soluble IL-2 receptor (sIL-2R) in SCZ, which has also been reported as associated with negative symptoms in chronic SCZ pathology (Akiyama, 1999, Sirota et al., 2005, Bresee and Rapaport, 2009). Upregulations in the sIL-2R is acknowledged as a marker of activation of the immune response, therefore may be indirect evidence of fluctuations in IL-2 concentration that may also significantly contribute to more severe negative symptomology. The binding of IL-2 to the sIL-2r can cause differential effects that exacerbate or suppress

immune responses further, based upon the cells that are involved (Damoiseaux, 2020). In SCZ, it is suggested that sIL-2R is significantly upregulated in first episode drug naïve patients, and that this may represent excessive activation of T-cells in early disease pathology (Upthegrove et al., 2014). Therefore, increased sIL-2r concentration may facilitate chronic neuroinflammation through IL-2 binding on immune responsive cells and the subsequent exacerbation of the immune response that is suggested as important for negative symptom presentation. However, findings based on the concentration and association of sIL-2r are somewhat inconsistent due to differences in population measured, medication status, demographic factors etc., and more research is needed to conclude the effects of IL-2. Changes in IL-2 concentration between early psychosis and chronic SCZ may be related to the 'state' cytokine description recently proposed for IL-2 (Capuzzi et al., 2017). All things considered, more research must be conducted to draw definitive conclusions about the contribution of IL-2 to symptomology, as current results are largely inconsistent both within and between disease stages in SCZ.

Anti-Inflammatory Cytokines and Negative Symptoms

In terms of anti-inflammatory cytokines, IL-4 and IL-10 were suggested to have a specific effect on the severity of negative symptoms in the FEP population. IL-4 was reported to display the highest overall correlation with negative symptomology across the review ($r = .67$, $P < .05$,) described in a study by Simsek et al. (2016). IL-4 is secreted by Th2 cells, microglia, and other immune cells, and plays a fundamental role in cell mediated immunity and tissue repair (Heeb et al., 2020). Typically, IL-4 is considered a neuroprotective anti-inflammatory cytokine that can function to silence IFN-γ and TNF-α. This routinely serves to downregulate the Th1 cytokine response, and also amplify Th2 based anti-inflammatory

activity, perhaps through interaction with central microglia and astrocytes to promote the neuroprotective phenotype (Gadani et al., 2012). Therefore, aberrations in IL-4 concentration may lead to defects in immune function within the brain and could contribute to the presentation of negative symptomology in FEP and SCZ. Simsek et al. (2016), reported no significant change in absolute IL-4 concentration as such, but noted a significant and positive correlation of IL-4 with the negative symptom subscale of the PANSS questionnaire in an adolescent FEP population. It is speculated that the ratio of IFN-γ:IL-4, as part of the Th1/Th2 balance respectively, may be important for the shift to TDO activation and the production of kynurenic acid by astrocytes within the CNS. In this instance, it could be speculated that elevated IL-4 concentration may increase the production of kynurenic acid through its' anti-inflammatory properties, possibly via the downregulation of proinflammatory markers that activate IDO in microglia, therefore shifting serotonin metabolism towards KYNA production (Muller and J Schwarz, 2010). However, this hypothesis contradicts the main body of evidence that suggests a proinflammatory environment in FEP and SCZ. Increased IL-4 concentrations may represent a more general marker of the inflammatory status in disease, rather than a direct mechanism of symptom emergence. Other research has drawn significant positive correlations between the shift in IFN-γ:IL-4 ratio in favour of IFN-γ production, which was subsequently correlated with IL-6, TNF-α concentration and KYNA production in medication naïve SCZ (Kim et al., 2009). This may suggest a dominant proinflammatory status in FEP that is important for the genesis of negative symptoms, and the increased central KYNA that has been suggested in SCZ. Though mechanistically different, both increased and decreased IL-4 concentrations may facilitate the production of kynurenic acid in SCZ, which ultimately fosters dysregulation of the glutamatergic and dopaminergic systems that are salient in negative symptom emergence (McCutcheon et al., 2019).

However, within the current review two out of three of the studies included (Haring et al., 2015, Noto et al., 2019), failed to find a significant correlation of IL-4 with the PANSS negative sub-score. Furthermore, evidence elsewhere suggests that overall, IL-4 concentration tends to remain relatively unchanged (Momtazmanesh et al., 2019), or even significantly decreased across FEP and more chronic exacerbations of SCZ pathology (Goldsmith et al., 2016), and evidence of the effect on negative symptoms is relatively scarce. Perhaps IL-4 represents a factor that contributes to effects on negative symptoms indirectly, through the inability to extinguish augmented proinflammatory action that is fundamental in SCZ pathology, leading to the more prominent increases in cytokines that are responsible for negative symptomology in FEP and SCZ. Alternatively, IL-4 has been suggested to prevent kynurenic acid formation through inhibitory action on the KAT2 enzyme of the tryptophan pathway, therefore reductions in IL-4 may indirectly promote kynurenic acid production and subsequent negative symptom emergence (Pedraz-Petrozzi et al., 2020). There does appear to be a link between IL-4 and negative symptomology, however further research is required to characterise the full extent of the effects of IL-4 in FEP and SCZ.

The cytokine that was linked most frequently with negative symptomology in the current review was IL-10. IL-10 is a potent anti-inflammatory cytokine that functions to oppose excessive inflammation and proinflammatory cytokines, and prevent cell damage in the brain and CNS after secretion by microglia and immune cells (Lobo-Silva et al., 2016). Studies investigating the concentration of IL-10 in the SCZ population appear to be somewhat inconsistent. Multiple different groups have reported increased, and decreased peripheral IL-10 concentration across FEP and chronic SCZ, which could in part represent differential effects of various cofactors including classification of disease, medication status, overall illness duration, comorbidities etc. (Momtazmanesh et al., 2019). Within the current review, two out of four studies reported a significant relationship between IL-10 and negative

symptomology in FEP. Xiu et al. (2014), reported a significant reduction in IL-10 concentration in first episode drug naïve patients, and an negative correlation between IL-10 concentration and the PANSS Negative sub-score, which remained significant after controlling for demographic factors. This is in line with another study that investigated IL-10 in the current review. Simsek et al. (2016), also reported a significant negative relationship between IL-10 and negative symptomology in an adolescent cohort with early onset SCZ, where lower peripheral IL-10 concentration was associated with a higher PANSS Negative sub-score. Indeed, research conducted elsewhere has suggested that dysregulation of IL-10 can alter the Th1/Th2 balance that may facilitate excessive inflammation (Murray, 2006). Therefore, these findings may suggest that the FEP population have a perturbed ability to mount an anti-inflammatory response to combat excessive inflammation, therefore shifting the balance of cytokines to a prolonged proinflammatory status, which may result in more severe negative symptomology (Xiu et al., 2014). Therefore, reduced IL-10 that is present in these two studies may represent a defect in the generation of an adequate anti-inflammatory response in the FEP population which could expose individuals to unprecedented inflammation and subsequent negative symptom emergence. This could lead to a vicious feedback circle of proinflammatory cytokines and immune cells that could manifest in the neuroinflammatory state that is characteristic of both FEP and SCZ.

However, randomised control trials conducted elsewhere somewhat contradict the aforementioned studies, and have suggested that IL-10 levels were increased in FEP and normalised after a course of antipsychotic treatment. In one study, the reduction in peripheral IL-10 levels was significantly correlated to improvements in negative symptomology ratings, therefore proposing that increased IL-10 may contribute to negative symptoms in SCZ (de Witte et al., 2014). This may expose IL-10 as a potential site of therapeutic action in order to reduce the severity of symptomology. Indeed, other groups have also suggested that IL-10 is

elevated in drug naïve FEP patients, and change in IL-10 concentration after treatment with antipsychotics were correlated with the changes in the PANSS Negative sub-scale, and draw on the potential of the variation in IL-10 to predict the change in PANSS Negative (Noto et al., 2015a, Noto et al., 2019). However, Noto et al. (2019), acknowledge that the accurate measurement of IL-10 concentrations in the blood may be difficult to establish, as this cytokine may be elevated in the presence of an immune challenge. Alternatively, elevated IL-10 concentration may represent somewhat of a compensatory mechanism whereby IL-10 is attempting to extinguish the exacerbated inflammation that is present in SCZ (Murray, 2006). Contrasting to these findings, the other two studies included in the current review that measured IL-10 observed no significant association with negative symptomology. Overall, IL-10 appears to be related in some way to negative symptoms and treatment response, although the direction and strength of this relationship remains unclear, as some studies only note a correlation upon the initiation of antipsychotic medication. Due to its' role in maintaining the balance in pro and anti-inflammatory cytokines, dysregulation of IL-10 may facilitate the general increase in proinflammatory cytokines that precede the emergence of negative symptoms.

Strengths & Limitations, and Future Directions

The role of cytokines in the pathology of FEP and SCZ is often difficult to define because of confounding factors including a longer duration of illness, antipsychotic medication, and associated comorbidities. Furthermore, FEP diagnosis and the classification of an individual as having 'first episode psychosis' as opposed to SCZ is often just a period of time. The pathologies are largely the same (Health, 2014). This makes drawing comprehensive conclusions regarding the role of cytokines during the development of FEP more difficult to

deduce. However, this systematic review utilised the operationalised definitions described by Breitborde et al. (2009), in order to classify an individual as within a period of 'first episode of psychosis'. This definition is based on objective criteria and is more rigorous and categorical than previous classifications (Breitborde et al., 2009). The setting of clear criteria for FEP diagnosis enabled the objective inclusion and exclusion of studies in this systematic review, for example the inclusion based upon disease duration, medication status, and absence of previous presentation to a clinical setting. This is a major strength of the current review. In addition, cytokine concentrations were converted to standardised mean difference within a random-effects meta-analysis, which negated any potential variance in the sensitivity of the assay used to assess each cytokine, as well as controlling for the inevitable heterogeneity that is present within this disease population. A limitation of the current review is the small number of studies eligible for inclusion in the meta-analysis, to examine the relationship between cytokine concentration and negative symptomology scale. Unfortunately, a lack of raw data reported for many studies and failed attempts to retrieve supplementary data due to lack of correspondence, meant that the relationship between cytokines and negative symptomology could not be assessed on a larger scale. However, this is discussed in a narrative context, and valuable insights on this relationship may still be drawn. Supplementary data was provided by two authors (Haring et al., 2015, Karanikas et al., 2017) which allowed greater insight into these studies in particular, to investigate the relationship between inflammation and negative symptoms in the FEP population.

Overall, the current systematic review demonstrated an increase in various cytokines in FEP, which provides further evidence for the inflammatory hypothesis of SCZ. Furthermore, the review revealed multiple relationships between different cytokines and negative symptoms as measured by the PANSS and SANS symptomology scales, and offered prospective mechanisms by which these effects may occur. Future research should seek to come to a

consensus about which inflammatory markers may contribute most to negative symptoms, and acknowledge a common method for cytokine quantification which will enable for higher quality comparison between studies. However, due to the heterogeneity that is present in SCZ as a disease group, it is difficult to determine the contribution of specific cytokines via single peripheral measurements that may be affected by short-term infection, medication, and comorbidities. Alternatively, future research may seek to conduct longitudinal studies that focus on patterns of cytokine change and how this may relate to specific negative symptom constructs, as this may be better equipped to draw conclusions about the impact of inflammation on the emergence of negative symptoms in SCZ (Goldsmith et al., 2016). It may be that longer periods of persistent inflammation are the driving factors for negative behaviours, rather than the effects of a given cytokine, the majority of which possess a multitude of pleiotropic effects. Particularly, future research should focus on the inflammatory profile of drug naïve FEP and 'at risk' patients where pathology is still in its infancy, and the full course and trajectory of negative symptoms can be better investigated. Further, A greater understanding of the potential contribution of inflammatory markers to the emergence of negative symptoms may be crucial in the production of effective therapeutic treatment for these symptoms in particular, which currently remain the most debilitating in long term SCZ pathology, predict poorer outcomes, and contribute to severe reductions in quality of life (Stahl and Buckley, 2007).

7.0 References

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